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ARNE MÜNTZING
Editor of »Hereditas»
Institute of Genetics, LUND, SWEDEN.

REDAKTIONSKOMMITTÉ

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SEED QUALITY AND THE PRINCIPLES OF FOREST GENETICS

By *C. EHRENBERG, Å. GUSTAFSSON, C. PLYM FORSHELL,* and *M. SIMAK*

GENETICS DEPARTMENT, FOREST RESEARCH INSTITUTE OF SWEDEN, STOCKHOLM 51

INTRODUCTION

SEED quality is a fundamental principle in all kinds of reforestation whether it occurs by natural reproduction, planned sowings or plantings. The population ultimately realized should react to soil, climate and management with a type of growth and development that is optimal under the offered conditions. This immediately emphasizes the genetical component of performance (ANDERSSON, 1955) but also the existence of ecological factors which influence plant and stand development. In addition, there is a distinct physiological component of performance, relating to the degree of development, maturity and healthiness of the seed used. Especially in northern countries, where flowering and cone formation are often sparse and seed development imperfect, this is a fact of considerable practical implication. Up to recent times, unfortunately, no satisfactory methods were devised, by means of which the conditions and status of the seed could be verified, quickly and in an exact manner.

This publication has as a chief aim to show that such an exact evaluation is technically possible. Moreover, it wants to stress that germination and juvenile growth are to a great extent dependent on the state of development of the seed, although as a matter of course genetic factors are also involved. In addition, it tries to indicate that environmental factors influencing seed development can be controlled by experiment and, finally, that a high quality of the seed according to genetic as well as physiological demands can be gained also in large-scale practical work.

I. SEED QUALITY IN ITS RELATION TO ENVIRONMENT AND HEREDITY

1. Seed development and X-ray diagnostics

Seeds of pine and spruce can be classified into five different embryo types (0—IV) and two endosperm types (A—B) by means of X-ray

photography (SIMAK and GUSTAFSSON, 1953 a, b, 1954), a method quick, inexpensive and reliable. The existence or abortion of the embryo, the occurrence of one or more embryos and their size as well as the size and absorption capacity of the endosperm serve as division principles.

0 — no embryo, no endosperm (»empty seed»);
I — endosperm, whether A or B, but no embryo;
II — endosperm (A or B) and one or several embryos, none of them longer than half the embryo cavity;
III — endosperm (A or B) and one incompletely developed embryo, one half to $^3/_4$ of the embryo cavity;
IV — endosperm (A or B) and one fully developed embryo, entirely or almost entirely filling the cavity.

When the endosperm is shrunk or less X-ray absorbing, indicated by the changed blackening of the photograpic plate, it is denoted as being of the B type. The weak X-ray absorption stands in relation to a destruction or an incomplete development of the endosperm (SIMAK and GUSTAFSSON, 1954, Fig. 19).

Embryologically type II is of special interest. The publications of BUCHHOLZ (1918, 1919; see also DAHLGREN, 1931) indicate that polyembryony is of frequent occurrence in *Pinus* species. It originates by two means: (1) the proembryo of one archegon divides into four embryos capable of development; also the so-called rosette cells give rise to embryo-like formations, these apparently degenerate; (2) several archegons are fertilized. By a combination of these two means a great many embryos may arise: if six archegons become fertilized up to 48 embryos, one half of which are capable of development. Usually most embryos are eliminated at an early stage. In X-ray diagnostics of pine and spruce no more than four embryos were seen in one seed at a time. ILLIES (1953) discovered in spruce up to five germinating embryos per seed. The degree of elimination is dependent on the type of climate and environment during the time of cone and seed development. Embryos, which originate by a splitting of the proembryo, are monozygotic. Embryos originating after the fertilization of different archegons and egg-cells, are polyzygotic. The participation of two (or possibly more) archegons can often be decided; in such a case two different embryo cavities are found.

Also in spruce these same embryo and endosperm types exist. The frequency of twin embryos is lower. They do appear, however.

In several hardwood genera, for instance *Alnus*, seed quality can easily be determined by means of X-ray photography (p. 323, Fig. 12).

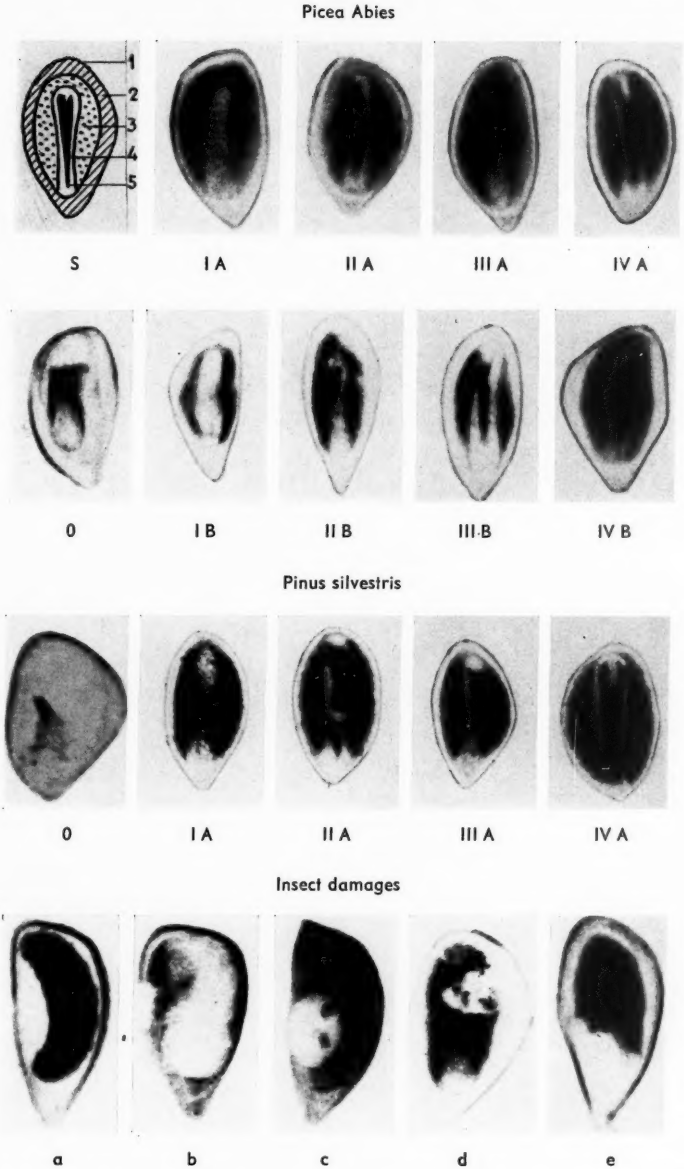
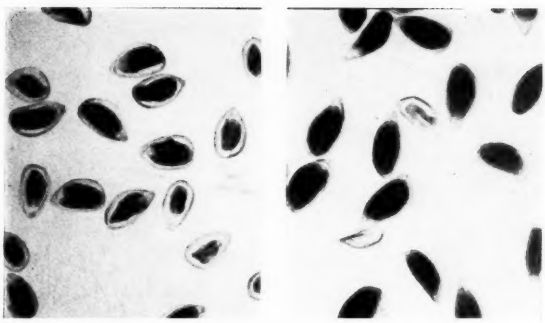


Plate I. Embryo types and endosperm in *Picea Abies* and *Pinus silvestris*. *Picea Abies*. Upper row: S. Schematic drawing of a full seed. 1. Seed coat, 2. Cavity between seed coat and endosperm, 3. Endosperm, 4. Embryo cavity, 5. Embryo, I A—IV A. X-ray-photos of embryo classes with endosperm A. Lower row: X-ray-photos of embryo classes 0—IV with endosperm B. — *Pinus silvestris*. X-ray-photos of embryo classes 0—IV with endosperm A. *Insect damages*: X-ray-photos of seed, damaged by insects, a. Larva of *Plemeliella abietina* SEITNER, b—c. Damage caused by the larva of a fly species, d—e. Damage probably caused by *Laspeyresia strobilella* L.



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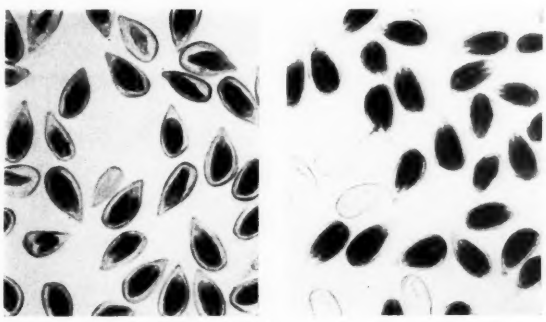

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Plate II. X-ray photography in *Picea Abies*. 1. Seed structure in a sample from Lapland: Hirvasrova, 67°15′, 550 m. Note the defect endosperm. 2. Skåne: Hallaröd, 50°55′, 94 m. Full endosperm. 3. Uppland: Bogesund, 59°24′, 30 m. Cones collected July 30, 1954. 4. The same. Cones collected September 8. Note the undeveloped endosperm in 3 as compared to the full seeds of 4, where embryo type IV A prevails. — Northern seeds remain at stages characteristic of early development in southern areas.

Plates I and II illustrate the various stages of seed development in pine and spruce.

2. Embryo development and type of pollination

PLYM FORSHELL (1953), as well as SIMAK and GUSTAFSSON (1954), showed that in pine self-fertilization leads to a good deal of seed abortion, expressed by the high amount of empty seed. In the spring of 1952 crossings and self-fertilizations were carried out in a series of selected plus and minus trees distributed over Sweden as the map in Fig. 1 indicates. Involved were the following types of pollination (Table 1):

(1) open pollination, the father parent is unknown;
(2) artificial crossings between strictly defined plus and minus trees of the same stand (intraprovenance crosses);
(3) crossings with plus or minus trees of different, more northern or more southern stands (interprovenance crosses);
(4) self-fertilization; and
(5) crossings involving different species.

Four hybrid combinations between *Pinus silvestris* as mother and *Pinus radiata, attenuta, Murrayana* and *densiflora* as fathers gave a few cones. Most of the extracted seed was empty: 96, 46, 55 and 97 % grains belonging to embryo type 0 as against 13—18 % after open pollination. The full seed, too, when formed is somewhat inferior, as especially seen in the crosses of *Pinus silvestris*×*densiflora*.

Controlled crosses within or between provenances produce seed material essentially similar in embryo type to the one obtained after open pollination. Clear-cut differences do not exist. In two trees (Y: 38^- and E: 1^+) the result is somewhat poorer with regard to the percentage of full-developed embryos (type IV). In five trees (S: 23^+, S: 21^+, Å: 3^-, E: 66^- and E: 67^-) the embryo quality of full seeds is slightly improved. There is no relationship of the embryo quality to the phenotype of the tree, whether plus or minus.

Distinctly different is the result after forced self-pollination. No complete self-incompatibility seems to exist in *Pinus silvestris,* although seed set is more or less decreased after selfing. With regard to seed quality there is a uniform increase of empty seed, in some cases with 50 per cent or more, as well as an obvious decrease of type IV. The four plus and the two minus trees arrange themselves in the following order: (1) with regard to the amount of empty seed: VIII: 1^+ > > Y: 38^- > S: 21^+ > VIII: 46^- > S: 23^+ > Y: 8^+, (2) with regard to the

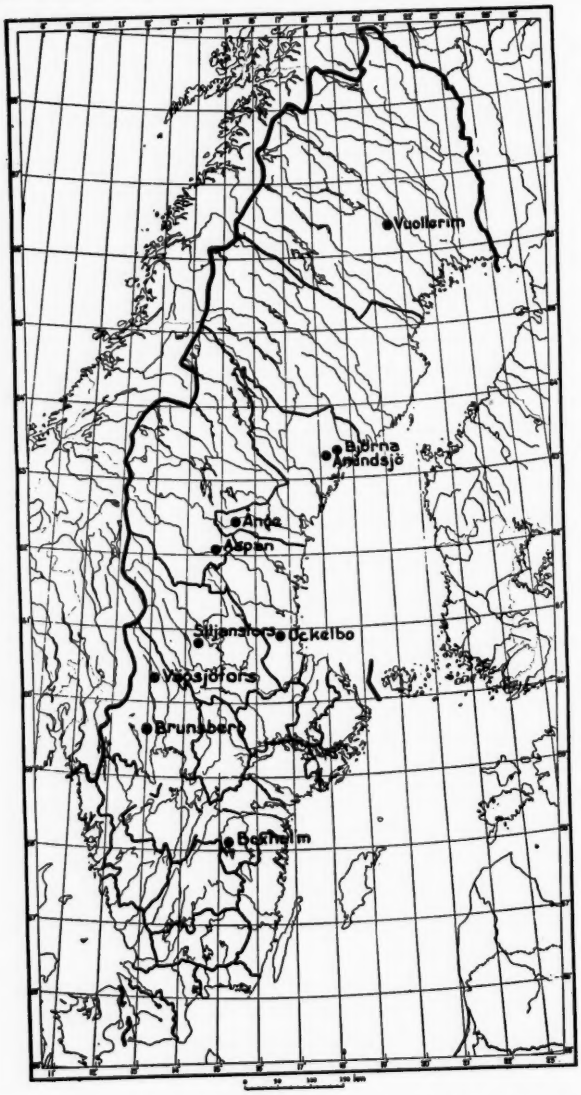


Fig. 1. The localities for mother trees of Scots pine specially selected for crossing purposes.

TABLE 1. *Embryo development and type of pollination.*

Locality	Type of pollination	Embryo type					No. of photographed full seeds
		0	I	II	III	IV	
		Per cent of total No. of seeds	Per cent of full seeds				
Boxholm	VIII: 1 open pollination	17,5	—	—	9,1	90,9	33
	selfed	78,4	—	1,7	18,6	79,7	59
	× *P. attenuata*	95,8	—	—	—	(100)	1
	VIII: 46 open pollination	13,0	0,2	2,3	6,0	91,5	650
	selfed	55,6	0,7	3,6	10,3	85,4	253
	× *P. radiata*	45,5	—	—	16,7	83,3	6
	× *P. densiflora*	97,0	—	20,0	20,0	60,0	10
	× *P. Murrayana*	55,0	—	—	—	100	9
Siljansfors	S: 23 open pollination	8,2	1,5	3,6	6,7	88,2	195
	selfed	48,7	1,6	6,4	7,2	84,8	251
	× S: 9	9,8	—	2,0	4,1	93,9	197
	× S: 21	11,1	0,4	3,8	4,6	91,2	238
	S: 21 open pollination	27,8	—	—	8,1	91,9	37
	selfed	61,6	0,8	0,8	13,3	85,1	128
	× S: 23	25,7	—	3,2	3,8	93,0	156
Aspan	Y: 8 open pollination	13,9	0,7	0,3	0,7	98,3	296
	selfed	21,6	0,8	0,4	4,1	94,7	266
	Y: 38 open pollination	3,1	—	0,3	4,0	95,7	300
	selfed	77,5	1,9	9,3	15,9	72,9	258
	× 11: 18	11,3	—	2,4	2,4	95,2	297
Ånge	Å: 3 open pollination	13,5	—	2,1	3,0	94,9	235
	× 11: 15	4,9	0,3	1,0	0,7	98,0	300
Vuollerim	E: 1 open pollination	4,0	—	—	0,5	99,5	200
	× E: 62	3,5	—	2,1	5,7	92,2	281
	× 11: 15	5,1	—	3,5	6,0	90,5	200
	E: 66 open pollination	5,8	1,0	3,1	2,6	93,3	195
	× VIII: 46	16,4	—	2,1	0,7	97,2	289
	× 11: 15	8,2	—	3,0	1,5	95,5	200
	× 11: 18	6,8	—	—	—	100,0	112
	× Y: 8	6,3	—	1,9	1,4	96,7	212
	E: 67 open pollination	10,9	—	3,6	7,7	88,7	194
	× VIII: 46	10,0	0,5	4,1	3,0	92,4	197
	× 11: 15	7,9	—	1,9	3,4	94,7	206

amount of embryo type IV: Y: 8^+ > VIII: 46^- > S: 21^+ > S: 23^+ > > VIII: 1^+ > Y: 38^-. The material of 1952, analysed by PLYM FORSHELL (*l. c.*) as to the quality of full seeds, gave the following order: E: 1^+ > > Å: 3^- > Å: 2^+ > Å: 1^+ > Y: 17^+ > 11: 18^- > 11: 15^+ > VIII: 46^- > > VIII: 2^+. The 1952 data lie on a much lower level of embryo quality than those of 1953, consequent upon the rougher climate in 1952.

Both in 1952 and 1953 there is a conspicuous formation of embryo type II after self-pollination, *i. e.*, the embryo development is arrested at an early stage. In consequence there is a higher degree of polyembryony. In every selected tree, also when embryo type II is entirely missing after open- or cross-pollination, it is formed after self-fertilization. In S: 23^+ and Y: 38^- of the year 1953 it amounts to 6 and 9 per cent, and in Å: 1^+, Å: 3^- and E: 1^+ of the year 1952 to 20 per cent or more. In the case of selfing the formation of a seed coat is initiated, also when no embryo develops or no endosperm is formed. When, on the other hand, embryos arise, they do not easily develop past the juvenile stage, rather they remain in the state of embryo types II or III.

This latter result is reflected in the low number of plants obtained after sowing the seeds produced by self-fertilization. PLYM-FORSHELL (*l. c.*) showed that in thirteen selected trees seeds sown in 1952 produced seedlings to a rate of $51{,}9 \pm 5{,}1$ % in the case of open-pollination as against a rate of $34{,}2 \pm 4{,}6$ % after self-fertilization. The difference reaches the level of significance with $P = 0{,}01$.

In this respect there is a significant difference between plus and minus trees. The open-pollinated seed of six plus trees gave an average germination in soil of 45 per cent as against 58 per cent in seven minus trees (PLYM FORSHELL, *l. c.*, Table 11). After selfing the difference is even more accentuated, namely 25 as against 42 per cent. This illustrates a phenomenon analysed in more detail in a following chapter (p. 339), *viz.* the occurrence of greater vigour in seeds from minus than from plus trees.

3. Seed development and climate

The harsh summer of 1952 resulted in a poor seed quality in Scots pine over large areas of Sweden (HUSS, 1952). South of latitude 62° N the germination ability was on an average satisfactory, but north of this boundary germination became more and more inferior with the increasing altitude and latitude. For a close analysis of this connection six special mother trees were selected (Fig. 2), chosen for study because

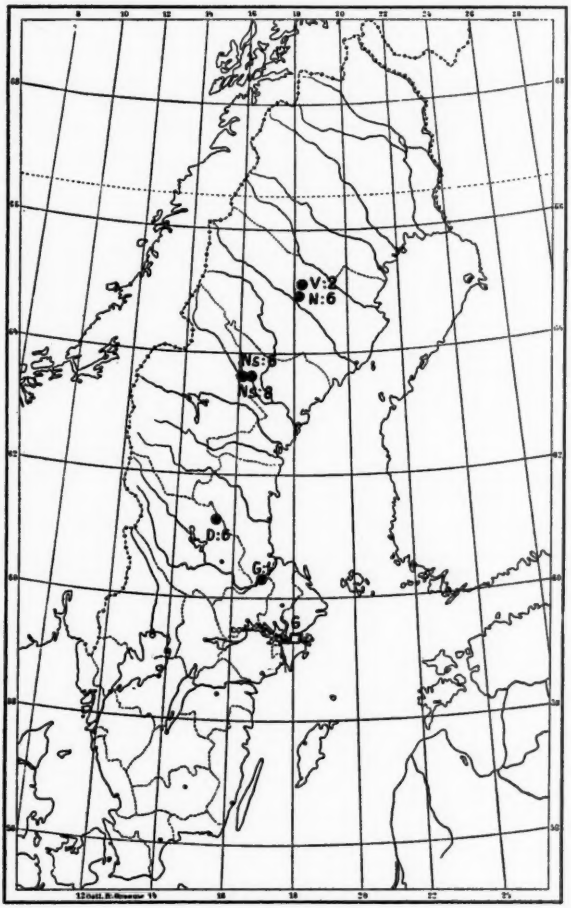
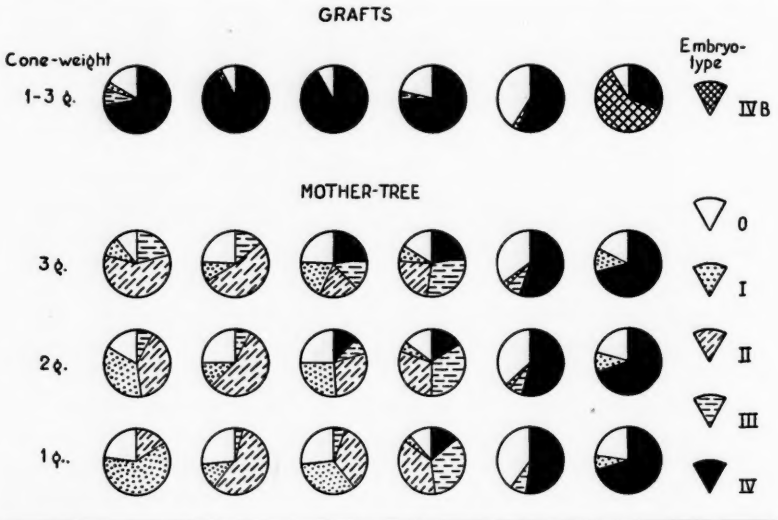


Fig. 2. The localities of six analysed mother trees and their grafts (G).

grafted clone individuals were available at the experimental station of Bogesund (G of the figure) and had produced ample cone and seed materials in 1952 (SIMAK and GUSTAFSSON, 1954).

There is a three-dimensional connection of cone size, seed number and seed size in *Pinus silvestris* (SIMAK, 1953 b). This implies that the seed size grows parallel to an increase in cone size and a decrease in seed number. For that reason when comparing seed development in

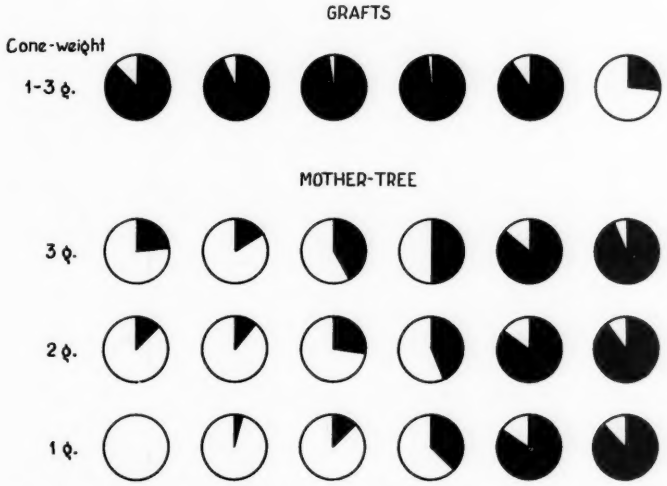


Genotype	V:2	N:6	Ns:6	Ns:8	D:6	G:1	G
Vegetation period	116	118	131		143	158	165
Latitude	65°6'	64°55'	63°35'		61°13'	60°15'	59°24'

Fig. 3. Climate, cone weight and embryo type in the six analysed genotypes of Fig. 2.

different trees and grafts it is necessary to fix a certain cone weight of the regression line for analysis. This has been done in Fig. 3. Here the climatic conditions of the mother trees and the grafts are specified as to latitude and vegetation period (the latter denoted as the number of days per year with a mean temperature of six centigrades or more).

Ignoring for the moment the embryo characters of the graft seed, we notice two fundamental properties: (1) In the northernmost trees V: 2 and N: 6 there occur no embryos of type IV; on the contrary, types 0, I and II predominate. On the other hand, in the two southernmost trees D: 6 and G: 1 embryo type IV prevails. The two trees Ns: 6 and Ns: 8 occupy an intermediate position. (2) In the four northern trees, where the embryo development is poor, the quality of the seed improves in the same degree as the cone weight grows, in this case from one up to three grams. This is especially obvious in the tree Ns: 6, where no embryos of type IV are present in one-gram cones but amount to more than 20 per cent in three-gram cones. In tree V: 2 there are 60 per cent



Genotype	V:2	N:6	Ns:6	Ns:8	D:6	G:1	G
Vegetation period	116	118	131		143	158	165
Latitude	65°6'	64°55'	63°35'		61°13'	60°15'	59°24'

Fig. 4. Climate, cone weight and germination capacity (calculated per full seed) in the six analysed genotypes of Fig. 2.

seeds of type I in one-gram cones but less than twenty per cent in three-gram cones.

Even better is this contrast illustrated with regard to the soil germination of the different seed samples of V:2 (Fig. 4). Seeds of one-gram cones do not germinate at all, those of three-gram cones to twenty per cent. In all trees, except in D:6, germination in soil is distinctly improved with the increase in cone weight. The conclusion is that in years like 1952, characterized by poor seed, there is no use collecting small cones of pine, at least not in northern areas, rather the collection work should concentrate on the largest cones available. The cone weight not only influences the seed yield and the average seed weight but also the embryo development and by that the subsequent soil germination.

That this influence is largely environmental in character, is obvious from the comparison with graft seed obtained at Bogesund. Disregarding the seed of tree G:1, which must be considered separately (p. 312),

Fig. 5. The distribution of pine trees analysed with regard to embryo development.

the seed quality and the embryo development are greatly improved by the transplantation to a more favourable climate. Notice, for instance, the high amount of embryo type IV in graft seed of tree V: 2, N: 6, Ns: 6, and Ns: 8. This seems to indicate that most or at least a great part of the variation in seed quality is really conditioned by the casual climatic conditions of the two years required for the formation of ripe cones in *Pinus*.

TABLE 2. *Embryo type IV in per cent of full seeds.*
(Within brackets the respective Nos. of samples.)

Latitude	Altitude, m							Total No. of samples
	0 — 100	100 — 200	200 — 300	300 — 400	400 — 500	500 — 600	600 — 700	
66—68°		95,5 (4)	81,8 (5)	47,7 (3)	43,5 (1)			13
64—66°	98,0 (2)	96,8 (2)	88,1 (9)	89,6 (7)	65,3 (2)	68,5 (1)		23
62—64°			97,7 (3)	95,9 (4)	86,0 (4)	75,5 (1)		12
60—62°	95,5 (2)	98,8 (2)	94,8 (2)	95,2 (3)	91,0 (1)	77,5 (2)	44,5 (1)	13
58—60°	97,6 (7)	97,4 (9)	94,0 (1)					17
56—58°	98,1 (4)	98,8 (2)	99,0 (3)	96,8 (2)				11

However, no doubt can appear that the genotypical constitution has some influence too on the seed quality. In tree D: 6, for instance, there is a high amount of empty seed; the proportion is approximately 35—40 per cent and largely independent of cone size and climate, as illustrated by the grafts, and distinctly higher than in the other five trees.

In the fall of 1953 an extensive study of seed development in Scots pine was carried out for a series of localities distributed all over Sweden (Fig. 5, MÜLLER-OLSEN and SIMAK, 1954). 89 trees were examined as to the percentage of empty seed, the embryo types of full seeds and the germination capacity in the laboratory and in soil. Table 2 gives a summary with regard to the amount of embryo type IV in percentage of full seed (not of total seed). The year 1953 favoured a good seed quality. Thus the consecutive years 1952 and 1953 contrast strikingly as to climate. The trees of V: 2 and N: 6 produced in 1952 no seed of embryo type IV whatsoever. In 1953 the lowest figure for trees of a region corresponding as to latitude (64—66° N) and altitude (> 300 m) was 78 % (in tree 22). Trees Ns: 6 and Ns: 8 gave a maximum figure of 40 per cent embryo type IV in 1952; in 1953 there was no lower figure than 90 per cent in the respective region.

Table 2 nicely illustrates the fact that at low altitude (0—200 m) embryo type IV predominated in 1953, irrespective of latitude (56—68° N). With a latitude of 60—62° N there is a decrease of seed quality at all altitudes above 500 metres. In the northernmost trees (64—68°) an inferior seed is formed even at an altitude of 200 metres. Consequently, the seed quality depends on the general weather of the years, but is in addition distinctly related to latitude and altitude. Moreover, there is a certain connection between empty seed and the percentage

of embryo type IV (calculated per full seed) as shown by the following extract of the data:

Empty seed % of total seed	0 — 10	— 20	— 30	— 60
Embryo type IV				
% of full seed	91,4	89,5	86,7	76,0
No. of trees examined	36	31	12	10

This indicates that a certain proportion of the empty seed owes its origin to environmental factors, influencing also embryo development of full seeds. Despite this there is now and then, as evidenced by tree D: 6, a special genotypical back-ground leading to the production of empty seed. In the material analysed by MÜLLER-OLSEN and SIMAK a wide range of empty seed was found, from 1 or 2 per cent in trees 20, 76 and 83 to circa 40 or 50 per cent in trees 2, 19, 49, and 56, but to what extent this is genotypically conditioned cannot be decided offhand.

4. Cone size and its influence on seed development

As previously emphasized, it was shown by SIMAK (1953 b), with example from two trees at Bogesund (provenance 59, trees 1 and 5), that there is a complex relationship of cone weight (size), seed size and seed number. The seed size can be expressed as a function of cone weight and seed number according to the equation $y=a+b_1x_1+b_2x_2$, where x_1 denotes the cone weight, x_2 the cone weight divided by the seed number, *i. e.*, the average cone weight required for the formation of one seed. a, b_1, b_2 are constants. In these two trees the cone weight exerted a greater influence than seed number on the seed size.

For the six selected trees V: 2, N: 6, Ns: 6, Ns: 8, D: 6, G: 1 the same relationship holds true (SIMAK and GUSTAFSSON, 1954, p. 35). It could be shown, however, that the seed weight of natural material was influenced by cone weight according to a gradually decreasing significance: Ns: 6***, G: 1***, V: 2***, N: 6**, Ns: 8**, D: 6*, and by the seed number (cone weight divided by seed number) according to the order: Ns: 8**, D: 6**, G: 1*, N: 6°, V: 2°, Ns: 6°. In all cases cone weight rather deeply influences seed weight, least in Ns: 8 and D: 6. In these two trees, on the other hand, the seed number has a marked effect. Apparently cone weight and seed weight act in a complementary fashion. In general, however, cone weight is the predominating factor. This was shown already in the discussion on seed quality and climate. Here, it was pointed out that small cones of northern trees, in certain years, gave

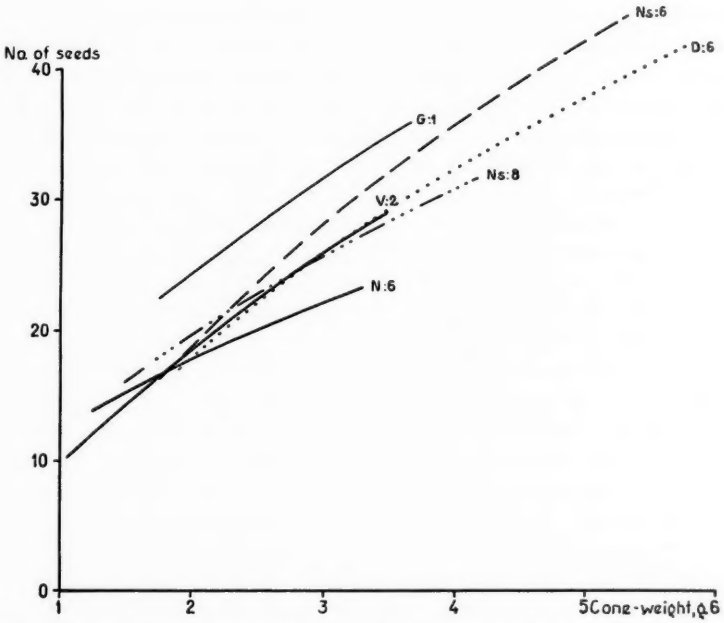


Fig. 6. Relation between cone weight and seed number of the six analysed mother trees in Fig. 2. Regression equation: $y=a+b\sqrt{x}$.

an inferior seed as to development, embryo type and germination. With a selection according to cone size embryo quality gradually improved.

As to the seed number of a cone, this also can be defined as a function of the cone weight. In this case the regression equation is most appropriately defined as $y=a+b\sqrt{x}$, where x is the cone weight and a and b two constants. Furthermore, the regression analysis proves that under normal conditions the six selected genotypes give clear differences with regard to the level of the regression curves ($P < 0{,}001$), but not as to inclination or degree of steepness ($P > 0{,}05$). (Fig. 6; *cf.* SIMAK and GUSTAFSSON, 1954, p. 32.) Three of the regression curves V: 2, Ns: 8, D: 6 have quite a similar progress. The curve of N: 6 lies largely below those of the other trees; in addition, its course is flatter. The highest general level of the curve is present in G: 1. Tree Ns: 6 has the steepest inclination of all trees and reaches the highest values of seed number.

Thus, the two trees G: 1 and N: 6 conspicuously deviate from one another. That this to a great extent rests upon differences in the genetic constitution, is vindicated by the behaviour of the graft material. Here, too, G: 1 has a distinctly higher average seed number than N: 6. On the other hand, the small noticeable differences between Ns: 6 and Ns: 8, although growing closely to one another in nature, most probably are environmental in character, since the seed number of their grafts is almost identical, when applying a fixed value of the cone weight.

The six analysed trees have a phenotypical character intermediate between plus and normal type. They were originally selected as plus trees but have not withstood the phenotype control. It is in this connection well worth examining the influence of plus contra minus type of growth on cone and seed properties. There is, as already considered by several authors, a remarkable correlation between the growth and quality of minus trees and their richness of flowering, size of cones and seed development. The rich flowering of minus trees has been specially pointed out by WETTSTEIN (1937). In addition, it is a well-known fact from the phenotype control of plus trees that many of these, especially extreme quality trees, flower rather irregularly and less abundantly than the neighbouring normal or minus trees. The correlation of minus type of growth and large cone size was stressed by PLYM FORSHELL (1953) in her study of self- and cross-pollination in pine. After her study was published more data have gradually accumulated. A statistical analysis of the trees involved in the country-wide crossing program gives the results shown in Table 3.

In twelve comparisons the minus trees surpass the plus trees as to cone size, giving P-values equal to 0,01 or less. In three cases they are slightly inferior, in two of these (Ockelbo: cross- and self-pollination) there is no significant difference, in one (Ånge: self-pollination) the P-value is close to 0,05. The lumped data go to show a most significant superiority of the minus trees with all three kinds of pollination.

A detailed analysis is worth while in this connection. Twenty-seven trees at seven localities were included in the study, fourteen of which are plus trees, thirteen minus trees. The averaged data irrespective of type of pollination (self-pollination for several reasons being excluded) and of year give the following sequence of decreasing cone size for locality after locality:

Boxholm: $46^- > 1^+ > 47^- > 2^+$
Ockelbo: $19^- > 1^+ > 15^+ > 18^- > 17^+$

TABLE 3. *Comparison of cone size in plus and minus trees of Scots pine.*

Locality	Type of pollination	No. of trees		No. of cones examined		Cone size (Mean)		t-value
		»plus»	»minus»	»plus»	»minus»	»plus» mm.	»minus» mm.	
Boxholm	open pollination 1949—1951	2	2	300	295	$32{,}1 \pm 0{,}23$	$34{,}4 \pm 0{,}39$	$6{,}002^{***}$
	cross » 1949—1950	2	2	355	200	$32{,}9 \pm 0{,}23$	$36{,}1 \pm 0{,}35$	$7{,}567^{***}$
	self » 1950, 1952	2	2	149	150	$32{,}1 \pm 0{,}39$	$35{,}8 \pm 0{,}52$	$5{,}765^{***}$
Ockelbo	open pollination 1950	3	2	150	100	$27{,}3 \pm 0{,}33$	$34{,}4 \pm 0{,}73$	$8{,}807^{***}$
	» » 1950—1952	1	1	150	150	$30{,}9 \pm 0{,}21$	$41{,}3 \pm 0{,}42$	$21{,}851^{***}$
	cross » 1950—1951	2	1	237	219	$28{,}9 \pm 0{,}34$	$28{,}4 \pm 0{,}26$	$1{,}268^{\circ}$
	self » 1951	2	1	97	50	$31{,}0 \pm 0{,}39$	$30{,}4 \pm 0{,}45$	$1{,}008^{\circ}$
Aspan	cross pollination 1950—1951	1	2	142	250	$27{,}2 \pm 0{,}42$	$36{,}1 \pm 0{,}31$	$17{,}104^{***}$
	self » 1950—1951	2	2	150	200	$29{,}4 \pm 0{,}49$	$36{,}6 \pm 0{,}33$	$12{,}138^{***}$
Ånge	open pollination 1948	1	1	42	50	$31{,}5 \pm 0{,}50$	$38{,}3 \pm 0{,}92$	$6{,}452^{***}$
	cross » 1948	1	1	69	77	$32{,}1 \pm 0{,}35$	$37{,}5 \pm 0{,}46$	$9{,}359^{***}$
	self » 1951	2	2	79	100	$39{,}6 \pm 0{,}43$	$38{,}3 \pm 0{,}45$	$2{,}093^{*}$
Björna	cross pollination 1951	1	2	74	62	$35{,}5 \pm 0{,}39$	$38{,}1 \pm 0{,}53$	$3{,}939^{***}$
Vuollerim	open pollination 1951	2	2	100	100	$31{,}0 \pm 0{,}59$	$35{,}2 \pm 0{,}61$	$4{,}986^{***}$
	cross » 1949—1952	1	2	378	843	$33{,}2 \pm 0{,}27$	$34{,}6 \pm 0{,}21$	$4{,}046^{**}$
	self » 1951	1	2	150	100	$34{,}3 \pm 0{,}28$	$35{,}4 \pm 0{,}57$	$1{,}725^{\circ}$

Siljansfors: $9^- > 21^+ > 23^+$
Aspan: $(19^+ >) 38^- > 33^+ > 37^- > 17^+$
Ånge: $4^- > 3^- > 1^+$
Björna: $5^- > 6^- > X^+$
Vuollerim: $67^- > 1^+ > 66^- > 62^+$

In every locality, the smallest cones belong to a plus tree, and in every locality but one (Aspan) the largest cones are found on a minus tree. The exceptional case, Aspan 19^+, is represented by no more than fifty cones obtained after open pollination in one single year. Of the other Aspan trees no data as to open pollination are available this year. The validity of the exceptional case must therefore be considered with some reserve.

The three-dimensional connection of cone size, seed number and seed weight will automatically — with a constant seed number — lead to a higher seed weight when cone size increases. The minus type of growth — whether hereditary or casual in character — will cause an increase in cone size (cone weight) and so indirectly also lead to a higher seed weight. Direct measurements support this conclusion. PLYM FORSHELL (*l. c.*, p. 14) gave the following data for the trees examined by her:

	Cone weight grams	1000grain weight of full seeds, grams
Plus trees	$2{,}64 \pm 0{,}14$	$4{,}22 \pm 0{,}15$
Minus trees	$3{,}36 \pm 0{,}19$	$4{,}85 \pm 0{,}07$
	$t = 2{,}98^{**}$	$t = 3{,}75^{***}$

After her study more elaborate work supports this conclusion. Using all available data on grain weight in the plus and minus trees, used for crossing purposes, we find the following data of 1000grain weights:

	Plus trees	Minus trees	No. of values	t
Entire material				
all trees, all years, all pollinations ..	$4{,}12 \pm 0{,}03$	$4{,}58 \pm 0{,}02$	71+88	$3{,}810^{***}$
Open pollination				
all trees, all years	$3{,}96 \pm 0{,}14$	$4{,}56 \pm 0{,}08$	32+39	$3{,}735^{***}$
Cross-pollination				
all trees, all years	$4{,}42 \pm 0{,}17$	$4{,}73 \pm 0{,}13$	28+37	$1{,}436^{0}$
Self-pollination				
all trees, all years	$3{,}81 \pm 0{,}16$	$4{,}18 \pm 0{,}15$	11+12	$1{,}676^{0}$

In all instances the minus values surpass the plus values. However, in the case of cross- and self-pollination the differences do not reach significance. In the case of open pollination, as well as in the entire material the P-values are below 0,001. A detailed study, of locality after locality, including samples of 200 seeds or more and values exactly comparable show the minus trees to be superior, too.

Boxholm: $46^- > 47^- > 2^+ > 1^+$
Ockelbo: $19^- > 15^+ > 1^+ > 18^- > 17^+$
Siljansfors: $9^- > 23^+$
Aspan: $38^- > 19^+ > 17^+$
Ånge: $4^- > 3^- > 1^+ > 2^+$
Björna: $5^- > X^+$
Vuollerim: $(1^+ >) 66^- > 67^- > 62^+$

The single exception from the rule that a minus tree has the highest 1000grain weight is thus afforded by the plus tree 1 of Vuollerim. However, the superiority of this tree is only apparent. A strict comparison of trees 1^+ and 66^- leads to the following division of the material:

	The weight of 1000 grains					
	Open pollination		Cross-pollination		Self-pollination	
Tree:	1+	66⁻	1+	66⁻	1+	66⁻
1949	5,45	5,13			—	4,42
1950	—	4,63				
1951	4,61[1]	4,71[1]	4,91[3]	4,95[3]	3,57	4,73
1952	5,89	—	5,93[1]	6,55[3]		

[1] Based on two samples. [3] Based on three samples. [4] Based on four samples.

If we exclusively use the pairs of values corresponding to one another as to grain weight, we obtain for 1^+ an average weight of 4,89 gram as against 5,21 for 66^-, *viz.* a distinct superiority for the latter.

Anyhow, for all localities studied the smallest 1000grain weight is found in a plus tree. On the other hand, the highest seed weight of a locality is at least in six out of seven cases connected with the minus type of growth.

5. Seed properties in mother trees and grafts of Scots pine (Pinus silvestris)

Grafting has become a fundamental tool of forest tree breeding. Scientific methods of clone propagation were first worked out in conifers

by SYRACH LARSEN (1934, 1937), although, of course, the method of grafting goes back to garden practice. The procedures are now as follows:

(1) One or more trees of a certain stand are selected for breeding purposes, according to standardized rules issued in 1950 by the Royal Board of Forestry.

(2) The selected trees are denoted as »plus trees», if they are superior to carefully chosen comparisons of the same stand in total production (»cubic plus trees»), quality (»qualitative plus trees») or to both kinds of properties (»entire plus trees»). Such a strict comparison forms the so-called phenotype control, which is now supervised by Mr. ENAR ANDERSSON, Stockholm. After its final approval the plus tree is given an official number, relating to the province where it grows (for instance, BD=the province of Norrbotten, AC=the province of Västerbotten, *etc.*, according to the letters for the registration of cars), and its owner (1000—1999, if it is owned by the Board of Crown Lands and Forests; 2000—2999, if its owner is a farmer; 3000—3999, if it is owned by a private company and is selected by the Association for Forest Tree Breeding; 4000—4999, also owned by a company but selected by the Society for Practical Forest Improvement; 5000—5999, if selected by the Forest Research Institute). For instance, plus tree BD 1151 of pine grows in the province of Norrbotten, latitude 60°00′50″, longitude 2°6′30″ east Stockholm, at an altitude of 235 metres; it has an age of 200 years as against an average age of 202 years for four selected comparable trees in its immediate surroundings, a height of 21 as against 17 metres, a diameter of 37 as against 27 centimetres; it belongs to the Board of Crown Land and Forests, is an entire plus tree, superior both with regard to production and quality.

(3) The approved plus trees are grafted at one or more institutions, will be tested in special clone trials, used for scientific experiments or take part in the scheduled clone orchards where high-bred seed is being produced.

These circumstantial procedures, including also scion collection and transport, illustrate the numerous possibilities of confusing the clones and the clone individuals before the time when they are planted in a seed orchard. For this reason, and in order to warrant the authenticity of the breeding materials, SIMAK (1953 a) made an elaborate study of seed morphology in *Pinus silvestris*, applying photographic methods. A similar analysis of *Picea Abies* is under way. Accepting his methods, PLYM FORSHELL (*l. c.*) analysed seed morphology in the trees selected for crossing purposes (Fig. 1) and SIMAK and GUSTAFSSON (1954) used the same and new principles for the identification of natural and graft seed in six different genotypes (Fig. 2).

In pine, as well as spruce, the seed characteristics are superior to any other kind of morphological properties in defining individual trees. Cone and needle characteristics are no doubt valuable, too, but they are more of an auxiliary help than decisive in identification. The seed characteristics can be grouped in the following way:

I. Properties of the seed itself:

1. The *size of the seed*, as determined by the absolute measurements, for instance, length or breadth.
2. The *shape of the seed*, defined as the indices of different absolute measurements, for instance, breadth/length.
3. Characteristic *details of the seed*, more or less specific for individual trees, for instance, a curved or a long fine-shaped micropylar tip; sharply marked, asymmetrically placed navel tap, *etc.*
4. The *structure of the seed surface*, which often is rather unique; with wart-like protuberances, a coarse granulation or a fine net-work; with a felt-like covering or a smooth glossiness.
5. The *seed colour*, varying from light-yellowish to dark-brown or black. It can, appropriately, be classified as to ground colour and surface pattern.

II. Properties of the wings:

6. The *wing shape*, long, narrow, slowly tapering towards the tip; short and broad; symmetrical or asymmetrical, with a rounded or a sharp tip, *etc.*
7. The *wing colour*, varying from yellowish white or faintly reddish to dark-red or black.

With the help of these properties it now seems possible to determine with full certainty whether a certain clone individual really belongs to a specified plus tree or is an admixture. In addition, by these means we can easily ascertain the phenotypical plasticity, the amplitude of variation of any one character in a changing environment.

The size of the seed easily changes, not only with the climatic conditions of the year but even with the difference in cone size, the number of seeds per cone and the position within the cone.

SIMAK (1953 a) divided the cone into ten sections (Fig. 7). In sections I and VII—X no seeds are generally formed. Most seeds are found in sections II and III and gradually decrease in number to section VI. In II and VI there are more empty than full seed, relatively seen, in III and IV rather the contrary. In addition, as seen from Fig. 8, the seeds are distinctly longer in sections III, IV and V than in II and VI. Between full and empty seed of *Pinus silvestris* there is no clear difference in size.

Similarly, as previously discussed on p. 302, SIMAK (1953 b) found that with a defined size and weight cones containing a high number of seeds

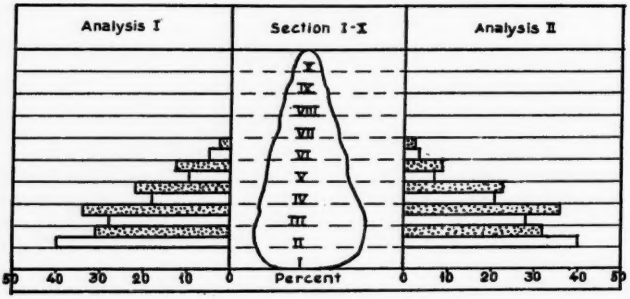


Fig. 7. Distribution of full (::::) and empty (▯) seeds in different sections within a cone of Scots pine. Values in % of the total amount of full resp. empty seeds.

form small seeds. When the seed number is low, the seeds are usually larger. In consequence of this relationship seed size can be expressed as a function of the cone weight and the quotient $\frac{\text{cone weight}}{\text{seed number}}$.

The influence of climate was analysed by SIMAK (1953 a, p. 21) in four different mother trees. With regard to seed area he found this property to decrease within the years examined in the following order:

Tree	No.	8: 8	Latitude:	67°51′	Altitude:	48 m	1948 > 1950 > 1949
»	»	8: 24	»	: 67°51′	»	: 48	»	1948 > 1950 > 1949
»	»	17: 25	»	: 66°02′	»	: 540	»	1948 = 1950 > 1949
»	»	18: 3	»	: 66°02′	»	: 650	»	1948 > 1950 > 1949

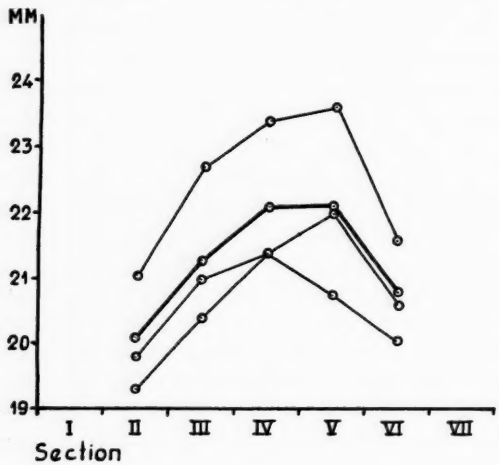


Fig. 8. Mean seed lengths in different sections of three cone sizes (faintly drawn lines). The strongly drawn line gives the mean values for the entire sample. 1 MM=0,2 mm.

i. e., in the genotypes specially examined there is a parallel pattern of variation.

In graft materials of northern trees, cultivated at Bogesund, the *1000grain weights* have increased considerably, owing to the more favourable climate of this experimental station:

Mother tree	Locality	1000grain weight, g	Locality of graftings	1000grain weight, g
V: 2	65°6′ 340 m.	3,220	59°24′ 32 m.	4,784
N: 6	64°55′ 320 m.	3,191	»	5,987
Ns: 6	63°35′ 225 m.	4,095	»	6,069
Ns: 8	63°35′ 235 m.	3,369	»	4,606
D: 6	61°13′ 250 m.	3,391	»	4,574
G: 1	60°15′ 80 m.	4,193	»	5,143

This comparison refers to the grain weights of entire seed samples, irrelative of the cone size. This is not strictly correct, since the cones of the graftings had grown larger than was the case under natural conditions (Fig. 9).

However, if we calculate for each cone of the graftings the corresponding value of the natural material, according to the regression equation $1000\,y = a + b_1x_1 + b_2x_2$, we find the following state of things:

In V: 2 the graft material has in thirteen out of sixteen cones a higher seed weight than that calculated for natural cones.

In N: 6 the seed weight of every graft cone is superior to the natural material.

In Ns: 6 this holds true in fifteen out of seventeen cones.

In Ns: 8 the graftings show higher seed weights in fourteen out of sixteen cones.

In D: 6 fifteen graft cones were examined. In all but two the seed weights are superior to the natural material.

The genotype G: 1 conspicuously deviates. Here, in spite of the superior grain weight of the entire sample, the seed weights of the graft cones when individualized lie below those calculated for natural materials in fifteen ouf of 22 cases. Owing to the fact that natural cones are rather small, graft cones on the contrary rather large, the seed

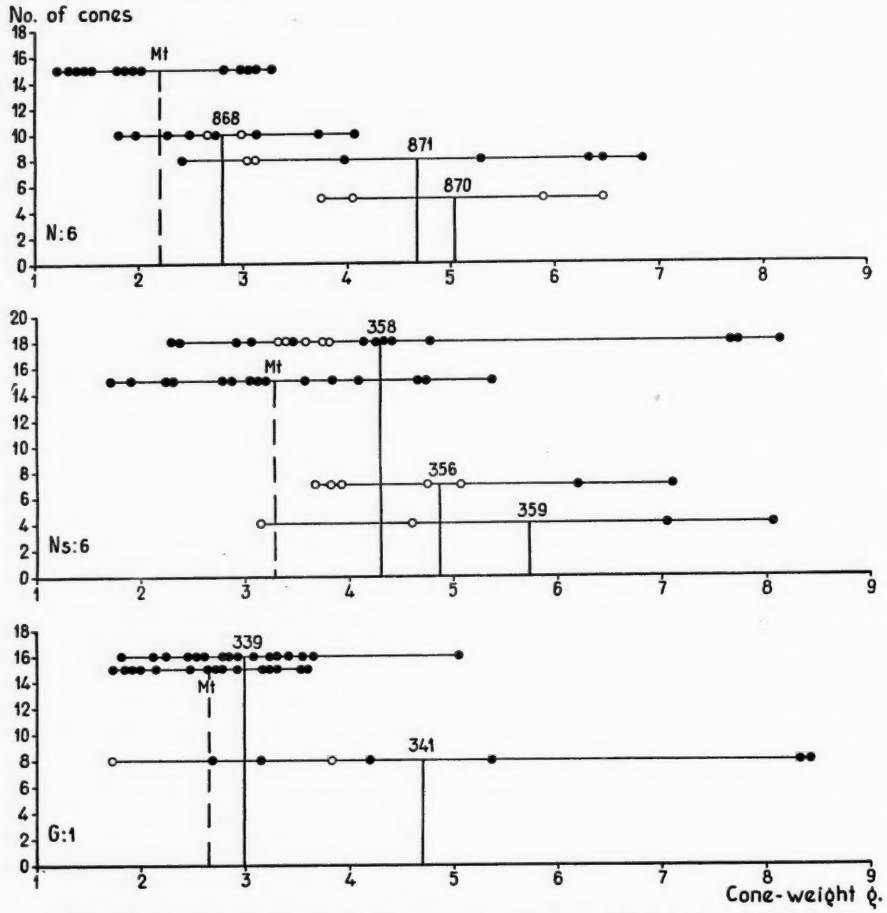


Fig. 9. Relation between cone weight and cone number in mother trees and grafts of specially selected genotypes (Fig. 2). The broken vertical lines denote the average cone weight of the mother trees, the entire vertical lines denote the cone weight of the grafts. (Mt=mother tree, figures=grafts.)

weight of the latter material when lumped will be high. The graftings of G: 1 were transplanted at the end of the vegetation period. In consequence, phenomena of a disturbed metabolism lead to a destruction of the endosperm. This fact was considered in detail by SIMAK and GUSTAFSSON (1954, p. 46).

Actually, JOHNSSON, KIELLANDER and STEFANSSON (1953) in their analyses of graft *contra* natural seed found the graft seed to have a 1000grain weight about 35 per cent higher than the corresponding natural seed and an amount of empty seed approximately ten per cent less. Moreover, the number of seeds per cone was increased by some 20 per cent. X-ray photography and regression analysis according to the methods used here suggest that these results depend on a better embryo development in graft materials as well as, generally, on the three-dimensional relationship between cone size, seed number and seed weight.

The seed colour varies less than the seed size but greatly changes with the degree of maturity. It is partially independent of cone size, number of seeds per cone and the situation of the seed within the cone. The type of climate profoundly influences the seed colour. This implies that the seed colour of a tree is rather uniform in a special year, even more so than many other seed properties, but that it often changes from year to year. The colour is an exquisite indicator of the degree of maturity. Usually, in seeds weakly developed, the colour is fainter than in fully ripe ones. Embryo types 0—II are in pine and spruce characterized by white, white-greyish or light-yellow tones of colour, having the micropylar tip slightly darker. This is in contrast to embryo types III—IV, which often possess a dark-brown or black-greyish colouring. Early statements by KUJALA (1927) agree with this result. Under good exterior conditions, consequently, the seeds become darkly coloured, under poor conditions they will be distinctly lighter. The light colouring of seed materials from high latitudes and altitudes is no racial characteristic but is actually caused by the poor maturity of the seed. If the seed of trees in favourable localities is prevented from full development, such a material will also become light-coloured. On the other hand, if graftings of northern trees are brought further south, for instance, as far as to Bogesund, the seed gets distinctly darker.

A few examples: In the tree denoted as V: 2 the natural seed has a yellow-brown colour, with a red-violet or brown margin; in the graft seed it is brown, like coffee, with a blackish-brown margin. In Ns: 8 the natural seed has a greyish-yellow tone, often with a somewhat darker shade covering the greyish yellow; the graft seed is blackish-brown to black. In D: 6 with its characteristic mouse-grey natural seed the colour darkens considerably in the graft materials from Bogesund. Also in G: 1, the southernmost mother tree of this investigation, the colour changes from light to darkish brown.

In spite of this conspicuous variation seed colour is, genetically seen, rather fixed. This becomes especially clear in years when the seeds ripen completely. The following analysis helps to clarify this point.

Genotype	*Ground colour*	*Surface pattern*
V: 2, natural seed	yellowish brown	spotted, in the periphery redviolet-brown
graft seed	coffee-brown	spotted, in the periphery black-brown
N: 6, natural seed	reddish brown	mosaic, cocoa-brown with colourless coarse grains
graft seed	yellowish brown	mosaic, dark-brown with uncoloured coarse grains
Ns: 6, natural seed	creamish brown	spotted, dark-brown
graft seed	dirtish brown	spotted, black-brown
Ns: 8, natural seed	greyish yellow	greyish black
graft seed	black-brown	black
D: 6, natural seed	mouse-greyish	spotted, dark-brown at the micropylar tip
graft seed	black-greyish	spotted, black at the micropylar tip
G: 1, natural seed	light brown	reticular pattern, black in the periphery
graft seed	dark-brown	the total surface is black

Outstanding is the mouse-greyish to black-greyish colour of genotype D: 6, as well as the reddish to yellowish brown colour of N: 6. A close inspection of the surface pattern helps to elucidate the genotypical influence.

Seed and *wing colours* change in a parallel way with the climate and the degree of maturity. Poorly developed seeds possess lighter tones of colour than fully ripe ones. The finding made by TUBEUF (1893) that the wing colour is conditioned by specific colour bodies of the dead cells of the wings is interesting. These colour bodies consist of gum-like substances, insoluble in water, alcohol, ether, potassium hydroxide or diluted acids. Under the microscope they appear like rounded brown to blackish lumps, increasing as to size and number, parallel to the colouring of the wings. The differences are especially striking when we compare natural and graft seed of northern genotypes.

The principles underlying *the seed shape* were originally worked out by SIMAK (1953 a) for pine and were then further analysed by PLYM FORSHELL (1953), SIMAK (1953 b, 1955), as well as SIMAK and GUSTAFSSON (1954).

SIMAK found that the seed shape is almost entirely identical in full and in empty seeds, as the following proportions of a specially examined tree go to show. (For the different denotions, *v.* Fig. 10.)

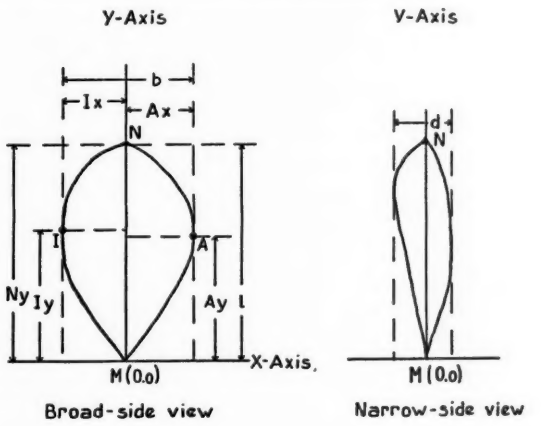


Fig. 10. Broad- and narrow-side view of a seed marked with the used denotions characterizing seed size.

Character	Full seeds	Empty seeds
b/l	49,7 ± 0,2	49,6 ± 0,6
d/b	62,6 ± 0,3	62,1 ± 0,6
Ay/l	57,4 ± 0,3	57,4 ± 0,3
Ax/b	58,4 ± 0,2	58,0 ± 0,4
Iy/l	53,9 ± 0,2	54,2 ± 0,4

SIMAK also examined the constancy of various indices for a sequence of years and detected no climatic influence. Similarly, the situation within the crown, the exposition, north *contra* south location, *etc.*, caused no clear variation.

There was, however, an influence of the position within the cone. This was especially noticeable with regard to the proportion of seed thickness (d) to seed breadth (b). The seeds of the basal sections have distinctly higher d/b-indices than those of the top sections. In addition, the asymmetry of the seeds (Ax/b) is less pronounced at the basis than at the top. Furthermore, the proportions of Ay/l and b/l vary considerably, the first one increases, the second one decreases with the distance of the seed from the cone basis. In general we may maintain that the narrow scales of the tip of the cone give rise to narrow seeds, and the greatly inclined scales of the cone basis lead to short and broad seeds.

In the six trees, frequently referred to (Fig. 2), a comparison between seed shape in mother trees and graftings was possible to carry through. Arranged according to diminishing breadth index (b/l) the values were:

	Ns: 8	Ns: 6	D: 6	G: 1	V: 2	N: 6
Mother tree ..	62,6 ± 0,9	58,6 ± 0,8	57,2 ± 0,6	55,5 ± 0,6	50,2 ± 0,6	49,3 ± 0,6
Grafting A ...	60,9 ± 1,3	57,7 ± 0,7	56,2 ± 0,7	56,1 ± 0,5	48,9 ± 0,4	51,3 ± 0,6
» B ...	61,2 ± 0,8	56,5 ± 0,7	55,5 ± 0,6	55,3 ± 0,7	48,9 ± 0,7	49,5 ± 0,7
» C ...	62,6 ± 0,7	56,5 ± 0,7				

The order of diminishing breadth/length index is for the mother trees — Ns: 8 > Ns: 6 > D: 6 > G: 1 > V: 2 > N: 6, for the graftings — Ns: 8 > Ns: 6 > D: 6 > G: 1 > N: 6 > V: 2, *i. e.*, entirely identical except for the two most northern trees with their poorly ripened natural seed. Here the order is reversed. For all genotypes the difference of mother tree and graftings is within the error. The analysed properties of asymmetry (Ax/b and Ay/l) give an exactly similar picture.

The surface structure, although no doubt strictly defined, has been examined in detail only with regard to the six genotypes V: 2 to G: 1. Here a close comparison was made between mother trees and graftings. In V: 2, N: 6, Ns: 6, G: 1 there is no difference whatsoever between the two kinds of seed material, even not so in the case of N: 6 possessing a seed surface especially characteristic, fine-granulate with solitary coarse grains intermixed. Deviating are the seeds of Ns: 8, where the surface of natural seed is lanate, but rough and glossy in graft seed with a mycelium-like uncoloured net of hairs, likewise the seeds of D: 6, where the surface of natural seed is lanate, too, but smooth in graft seed. The occurrence of the wooly structure is probably connected with the degree of seed development and maturity.

Nor has the property of *wing shape* been analysed to any great extent. As pointed out already by WETTSTEIN (1948), it is fairly uniform within any one tree. However, like the seed shape it changes with the position within the cone: it is more elongate at the tip of the cone than at the base. Generally said, it varies more than the seed shape. As in the seed itself the properties of the wing can be divided into absolute dimensions, proportions and specific details. The variability is wider with the absolute dimensions, much less with regard to proportions and details.

Most noteworthy is the characteristic difference in the wing properties of the mother tree G: 1 and its graftings. The wings of natural seed are knife-like, rather narrow at the base; in graft seed of this genotype they are sharp-tipped and distinctly broadening towards the base. In the five other genotypes (V: 2 to D: 6) the wings are exactly similar in graft and natural seed. The deviating behaviour of genotype G: 1 is certainly related to the late transplantation of the graftings.

SIMAK (1953 a, p. 22) closely studied the constancy of distinct *seed details* in two plus trees (Boxholm 1^+ and 2^+). Both trees are distinguished by the shape of the micropylar tip. In tree 1 this is straight and needle sharp. In tree 2 it is bent, almost hook-like. He also studied the influence of the type of pollination on these seed details and found their constancy to be high:

		Micropylar tip			
Boxholm 1⁺	Exactly identical	Similar	Clearly different	
Open pollination	18	2	—	=20 seeds
Cross » (+×+)	20	—	—	=20 »
» » (+×—)	18	2	—	=20 »
Self »	17	3	—	=20 »
Boxholm 2⁺				
Open pollination	15	5	—	=20 »
Cross » (+×+)	14	6	—	=20 »
» » (+×—)	20	—	—	=20 »
Self »	6	—	—	= 6 »

As shown by the table, the non-identical seeds do not really deviate, although the characteristics are not fully developed. Still, with regard to this specific feature the difference between the trees is so striking that it is possible to separate the two kinds of seed within a mixed sample.

Similarly, there is an almost complete identity as to seed details in full *contra* empty seeds, in large *contra* small cones, in different parts of the cone and in different years.

Of the six specially examined genotypes V: 2 to G: 1 some have quite singular seed details. In N: 6 the border-line of the seed running from the micropylar tip to the navel is very pronounced, causing what looks like a double navel. This property remains identical in graft seed. In addition, it is independent of embryo type (0—IV). Ns: 6 has a greatly rifled surface, which is as pronounced in graft as in natural seed. In D: 6 the navel tip is broadly rounded and the border-line between the micropylar and the navel region sharply marked. No difference was to be seen in the two kinds of seed. G: 1 has a long sharp-pointed tip of the seed, exactly similar in natural and graft seed. Also in V: 2 and Ns: 8 some minor characteristics define the genotypes.

This analysis has proved, we consider, that there is a remarkable multiformity of seed details, these, moreover, keep almost entirely constant from year to year, from cone to cone, from mother tree to its graftings, *etc.* Applying, in addition, properties of seed and wing shape,

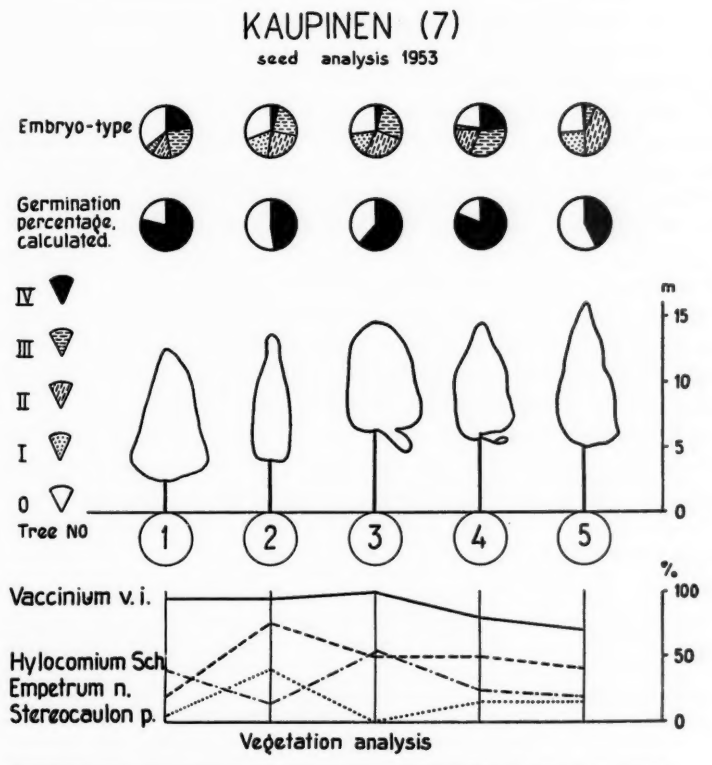
surface structure, we may state that every clone individual used in plant breeding and genetical experimentation can be adequately controlled. Mixtures and confusions are easily corrected. Plant breeding work can be carried on in a completely clean fashion.

This rather tedious comparison of seed shape and seed details in natural and graft seed was originally caused by the finding in 1953 that one grafting of mother tree Ns: 7 gave cones that appeared different from the ones produced in the sister graftings. Clone individual 47—392 possessed smaller, more compressed cones with more flattened apophyses than clone individuals 47—393 and 394. In addition, the colour of the young cones differed, being almost cocoa-brown with red stripes of anthocyanin, in contrast to the rather uniform moss-green colour of the cones of the sister graftings. The difference gradually became smaller towards the end of the vegetation period. However, just the cones were dissimilar, no difference whatsoever with regard to seed characters was traceable. In this case we have reason to conclude that the extraordinary state of things was caused by casual disturbances of the normal metabolism during the time of cone maturity. In every case where a more searching analysis of specific cone and seed characteristics could be performed, involving a specified mother tree as well as its graftings, a complete identity was found.

6. Embryo development in an Arctic population of Scots pine

The new provenance studies of North European *Pinus*, begun at the Genetics Department in 1948 and the first phase finished in 1954, involve 2500 different mother trees (genotypes) and their progenies, representing 112 different stands (populations). A great many mother trees were grafted in 1952—1954, and the grafting work is gradually carried on. The progeny tests are performed in 29 different places distributed all over Sweden. In five more progeny tests the resistance against *Phacidium infestans* is being examined. The provenance work was largely planned and guided by Messieurs LANGLET and EICHE. The testing of progenies and graftings is amplified by studies of seed development in the mother trees, their growth and branching habit, as well as their morphological characteristics, ecology and microclimate, *etc.*

Some provenance stands are situated not very far from the tree line, at high latitudes and altitudes. These are of special interest for the future reforestation of the northern areas now being barren or of no or little economical value (HÖJER, 1954). Plant breeding has here enormous



Age	150	109	170	155	160
Diam.mm (at 1,3m)	342	227	364	296	344
Height, m	12,5	13,5	14,5	14,5	16,0

Fig. 11. Seed analysis of five genotypes in an Arctic population of Scots pine, as well as tree characteristics and vegetation analysis (two phanerogams, one moss species, one lichen).

objects in creating new populations, characterized by a vigorous growth and a reliable cone and seed setting also under seemingly poor conditions. Selection work, as it is now performed (p. 308), largely concentrates on the growth properties and ignores seed quality and embryo development. As repeatedly pointed out in the preceding pages, the climatic conditions greatly influence seed quality, in such a way that the better the climate the more seed is referred to embryo types III

and IV. This fact does not exclude the possibility that there exist genetical differences of seed development as to its tolerance to harsh exterior conditions, low summer temperatures, frost and frozen soil, cold winds, *etc.*

One stand included in the mentioned progeny tests is denoted as provenance No. 7. It lies north of the Arctic Circle at a latitude of 67°51′ and at an altitude of 355 metres, locality Kiruna: Jukkasjärvi, Kaupinen. The stand has at its disposal a vegetation period consisting of almost exactly 100 days. Unfortunately, the trees selected for progeny tests do not in this case lie in the immediate vicinity of one another. This was owing to the difficulties of harvesting enough cone and seed materials in 1948—49. However, five trees depicted in Fig. 11 grow close enough and under similar ecological conditions, recently analysed by Messieurs EICHE, SIMAK and MÜLLER-OLSEN. They permit a reliable comparison. This is especially true of the trees denoted as Nos. 4 and 5. These have almost the same age, 155 and 160 years, respectively, and their microclimate is, according to all evidence, closely similar. One of these two trees (No. 4) gives the best seed quality of all five trees considered, and the other one (No. 5) the poorest seed quality. The data run as follows:

	Embryo type, %					Calculated germination in the laboratory, %	
Tree No.	0	I	II	III	IV	All seed (0—IV)	Full seed (I—IV)
4	22,1	1,1	16,1	37,1	23,6	64,1	82,3
1	33,7	3,3	14,6	26,2	22,2	52,4	79,0
3	28,8	10,4	28,4	24,9	7,5	43,7	61,4
2	29,9	16,9	27,0	21,2	5,0	37,2	53,1
5	26,4	23,8	38,0	11,2	0,6	29,8	40,5

There is a striking contrast in behaviour between trees 4 and 5. In fact, tree 4 has almost fourty times as many embryos of type IV as has tree 5, and five times as many embryos of types III and IV combined. Suggestive is, moreover, the almost entire absence of embryo type I (endosperm but no embryo) in tree 4 and the abundance of such seed in tree 5.

Certainly, there may exist unknown factors causing different microclimates of these two trees. However, there is no reason to postulate a greater difference between these two trees than the more obvious difference in ecology between trees 1, 2 and 3, on one hand, and trees 4 and 5, on the other. Taking into account that the cone formation was for two subsequent years decidedly less in tree 5 than in tree 4, we conclude

that tree 5 is well adapted to the climate vegetatively but less so in generative respects. Tree 4 is vegetatively, too, fairly well adapted and decidedly so in generative respects. To all evidence this difference in behaviour is genotypically conditioned. When erecting new stands in the north, two methods are available: (1) the establishment of special clone plantations for seed and plant production, relying on selected trees, satisfactory under natural conditions not only with regard to production and quality but, in addition, *well adapted as to seed set and embryo development*; (2) the application of methods of natural reproduction; *in such a case the seed trees should be controlled not only as to growth and quality but also as to cone formation and embryo type.* When building up adequately a new population according to the first method, the next generation should be capable of natural reproduction, too, when this is, on the whole, possible for soil-technical or other reasons.

In this connection it is well worth mentioning an idea originally put forward by HESSELMAN in 1907. He pointed out that in northern stands there often appear single individuals of pine, characterized by their unusually rich seed production. He considered these trees to possess a special type of branching and crown formation, different from that of normal *lapponica* pine. He also puts forward some directions for their employment in forestry. Let us start with the realization of »sex-potent» individuals, he says (p. 80). In their progeny those trees should be kept which look like the mother trees. The other ones are cut. This is an easy task, since the »sex-potent» individuals are easily distinguished owing to their dark-green needles. If we want to produce timber, based on the seed from the potent mother trees, the progeny ought to be handled in just the opposite way, *i. e.*, the »sex individuals» should be cut and the productive and qualitatively good trees be left.

This method overemphasizes the correlation of morphological properties and the generative or, as the case may be, the vegetative capacity — »dark-green needles and high seed set». Moreover, the correlation has not been verified in later work. Despite this, we now possess a photographic method of controlling the embryo development in an exact way. By this method a positive selection, both as regards production and seed set, can be carried through. The negative correlation of vegetative growth and generative capacity does not appear to be so strong that these opposite qualities cannot be united or, at least, kept in good balance.

The intentional production of new ecotypes for high latitudes and altitudes involves, in addition to progeny tests and growth control of the mother clones, also a study of genetical adaptation to the extreme type of environment with regard to embryo and endosperm development.

7. X-ray photography and seed setting in Alnus

Previously a strict analysis of seed quality was rather difficult in Swedish alder species. The method of fanning the seed, in order to separate full and empty grains, did not work efficiently, since the fanned sample of full seed always contains more or less empty grains and that of empty seed a certain amount of full grains. However careful the fanning, even repeated, it never gives the same successful result as in pine and spruce. In Table 4 this difficulty has been illustrated. First, the seeds were separated by careful fanning into a »full» and an »empty» group. From each of these two groups a random sample of 100 seeds was then diagnosticized by X-raying. The failure of the desired separation was immediately revealed. No less than 30 per cent empty grains were present among the full ones. A similar figure of contamination was obtained with regard to the »empty» sample. As seen by the figures, there are certain differences in separation owing to the origin of the material. Samples 10 and 40, for instance, still include 70—80 % empty grains in the »full» group but the »empty» one is almost completely clean. Samples 2, 3 and 29 have rendered good results with regard to full grains, which are almost devoid of the empty

TABLE 4. *Seed analysis in Alnus glutinosa by fanning and by X-ray photography.*

Sample No.	No. of »full» seeds after fanning		No. of »empty» seeds after fanning		Per cent full seeds (X-ray analysis)	Per cent of embryo type		No. of seeds classified	Per cent twins
	By X-ray photo		By X-ray photo						
	full	empty	full	empty		II + III	IV		
2	91	9	78	22	84,5	9,47	90,53	169	1,8
3	91	9	56	44	73,5	10,88	89,12	147	1,4
8	80	20	35	65	57,5	7,83	92,17	115	4,3
10	29	71	2	98	15,5	9,68	90,32	31	3,2
11	84	16	63	37	73,5	5,44	94,56	147	2,0
14	72	28	30	70	51,0	17,65	82,35	102	4,9
15	66	34	20	80	43,0	5,81	94,19	86	0,0
21	58	42	13	87	35,5	8,44	91,55	71	0,0
29	92	8	63	37	77,5	3,25	96,75	154	0,0
40	18	82	1	99	9,5	15,79	84,21	19	0,0
Average	68,1	31,9	36,1	63,9	52,1				

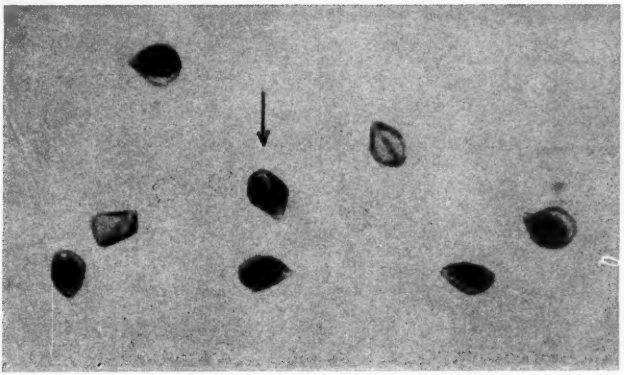


Fig. 12. *Alnus glutinosa.* X-ray photo of full and empty seeds. Seed with twin embryo marked with an arrow.

intermixture. Here, on the other hand, the empty group is heavily contaminated by full seeds.

In certain samples the occurrence of twin embryos was revealed by means of X-ray photography (Fig. 12). There is a concentration of di-embryonic seeds to certain genotypes, for instance trees 8 and 14.

All provenance material averaged, there is approximately half the seed material full and the other half empty, actually 52 % full as against 48 % empty seeds. Of the full seeds 82 to 97 % belong to the highest embryo class, approximately corresponding to type IV in pine and spruce. Since no endosperm is present in *Alnus* seeds, there is no possibility of distinguishing between A and B seeds according to a difference in endosperm.

X-ray diagnostics have made possible a more detailed study of crossability in *Alnus*. There are two species native to Sweden, *A. glutinosa* and *incana*, both diploid with $2n=28$. Hybrids and back-crosses have been postulated to occur by taxonomists, some even north of the distribution area of the southern species (*A. glutinosa*). Apomixis has been found in foreign species (*Alnus rugosa*; *v.* GUSTAFSSON, 1947). A study of crossability within the genus therefore implies an interesting program (EHRENBERG, 1946). The experiments reported here refer to one year only (1954), although EHRENBERG has continually carried out crossing work since 1942. The studies were performed in the greenhouse, involving two- to four-year-old graftings. More than 3000 pollinations were made in 1954. 84 of them are of interest in this connection. They produced a large

number of seeds, 12.000 of which were X-ray photographed. These represent seven trees and three provenances of *Alnus glutinosa* and six trees and four provenances of *Alnus incana*. The following table gives the results.

	Total number of combinations				
	No cones formed	Cones with empty seeds	Cones with one to several full seeds	Per cent full seeds	Per cent embryo type IV
A. glutinosa					
Intraspecific crosses	1	2	6	$\frac{320}{1482}$ = 21,6 %	18,6 %
Self-pollination	0	0	3	$\frac{74}{1330}$ = 5,6	4,7
×*A. incana*	4	21	5	$\frac{68}{1292}$ = 5,3	4,7
Control	1	5	1	$\frac{2}{236}$ = 0,8[1]	0,8[1]
A. incana					
Intraspecific crosses	2	0	3	$\frac{153}{576}$ = 26,4	24,0
Self-pollination	0	1	4	$\frac{167}{1866}$ = 9,0	6,4
×*A. glutinosa*	9	1	11	$\frac{269}{4919}$ = 5,5	4,5
Control	3	1	0	$\frac{0}{401}$ = 0,0	0,0

[1] Contamination.

The control material unfortunately comprised one unsatisfactory bagging, resulting in two full seeds, as against circa 1000 in the artificial pollinations. Cones are formed in the successful control material, too, indicating the presence of *parthenocony* (SÖDERBERG, 1953). This implies that they arise even without any pollination. In addition, seeds may form, which are then empty (disregarding the two full seeds of *A. glutinosa*, control). It is also evident that selfing leads to a high abortion in both species, no more than $^1/_{20}$ to $^1/_{10}$ of all seeds containing an embryo. Species crosses are approximately to the same extent successful, at least in these experiments where graftings are used as crossing partner. A detail study shows that the success or failure of species crossings is connected with the genotypical constitution of the parents. Some genotypes are distinctly more apt than others to the production of hybrid seed. A similar state of things was pointed out above with regard to the formation of twin embryos. Finally, we

conclude that apomixis of the autonomous type cannot be present in the material analysed.

II. SEED QUALITY AND ITS INFLUENCE ON GERMINATION AND PLANT VIGOUR

1. Seed quality and germination in pine and spruce

A series of experiments were undertaken in order to test the reliability of embryo and endosperm development in conifers as a quick method for determining germination capacity. Certainly up to now the methods are applicable only to fresh seed. Investigations under way indicate the possibility, however, of judging from the X-ray absorption of stored old seed its capacity of germination. Damaged seed, whether the damage is due to rough extraction, dewinging or insect attacks, can be defined rather accurately (Plate I; *cf.* also MÜLLER-OLSEN and SIMAK, *l. c.*, Fig. 5 A). Seed damaged physiologically, in our case owing to a late transplantation of graft material, can as evidenced by the discussion in SIMAK and GUSTAFSSON (1954) be detected by the conspicuous decrease in X-ray absorption (*l. c.*, Fig. 19).

With regard to the six selected pine trees studied by SIMAK and GUSTAFSSON (*l. c.*) there was a clear-cut correlation of embryo quality and germination in soil. They found the following figures of soil germination after thirty days:

Embryo type	0	I	II	III	IV
Germination	—	(5)[1]	13	63	94 %

[1] Misclassified seed.

The analysis shows that embryo type IV ensures an almost complete germination. This is distinctly inferior with embryo type III, and even more so with embryo type II. In this connection it is well worth mentioning that great part of the northern pine seed harvested in 1952 gave a germination rate of 10—20 per cent indicating a high proportion of embryo type II.

More detailed studies were performed by MÜLLER-OLSEN and SIMAK (*l. c.*). They compared the germination capacity of embryo types I—IV and endosperm types A and B under good laboratory conditions as well as in soil cultures of varying severity. Here the influence of endosperm quality was studied more closely.

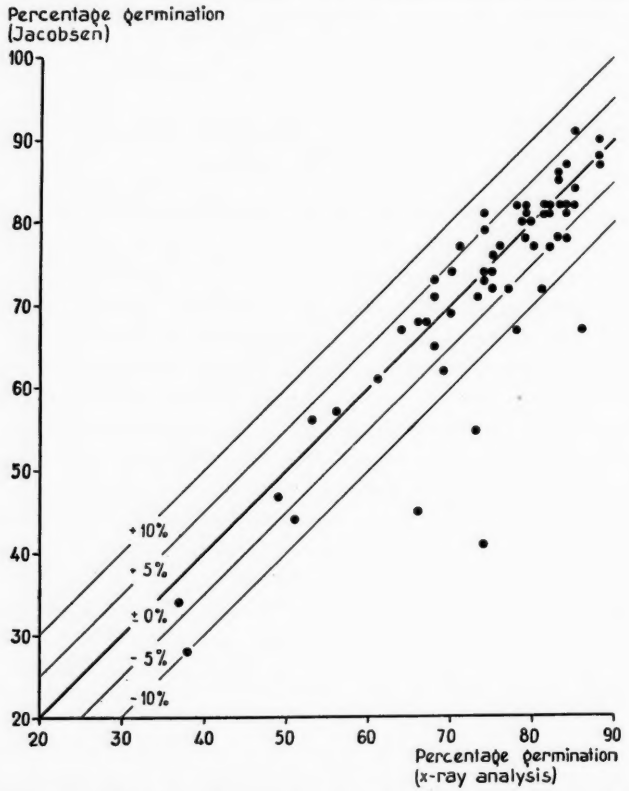


Fig. 13. The connection of found germination rate (Jacobsen) and calculated germination after X-ray analysis of embryo type (endosperm type not considered). Deviating values concern samples with a defective endosperm. Analyses performed independently in the Jacobsen germinator at the Departments of Forest Regeneration and Genetics. Material: Scots pine (1954).

Embryo type	0	I		II		III		IV		
Endosperm	—	A	B	A	B	A	B	A	B	
Germination in the laboratory I a	—	(0)	—	36	—	81	—	98	—	%
b	—	(1)	—	50	—	88	—	99	—	%
Germination in the laboratory II ..	—	—	—	46	5	83	43	96	68	%

I: Entire material of 89 trees, a: within 10 days, b: within 30 days.
II: Samples of four northern trees, within 30 days.

As in the previous analysis fully developed seeds (type IV A) germinate almost 100 per cent, seeds of type III A to circa 85 per cent. Half

of the seeds belonging to type II A are incapable of germination, even under the best laboratory conditions. The occurrence of a B instead of an A endosperm greatly reduces the germination capacity: in embryo type IV with 28 %, in III with 40 % and in II with 41 %. Seeds of type II B scarcely germinate at all in the Jacobsen germinator. Under harsh exterior conditions, of course, they germinate even less.

The year 1954 was characterized by a profuse flowering and cone setting in spruce (*Picea Abies*) all over Sweden. Owing to the more than ten year lapse since the preceding good seed year, an intense collection work has set in. A study is under way of correlating the embryo and endosperm development in spruce to the germination rate and the vigour of the seedlings resulting. Offhand, of course, there is no certainty that the connection is so intimate and strong in spruce as in pine. It may be possible that exterior conditions modify the response in an entirely different way.

For this reason a proposal by Mr. T. ANDRÉN, Forester of the Mo and Domsjö Company, was accepted implying that the quality of seed in some stands of spruce from northern Sweden be examined by means of X-ray photography. Parallel to our study, and completely independently, a study of germination ability was carried out by Mr. E. STEFANSSON of the Forest Tree Breeding Association, substation Sundmo.[1]

In the first set of analyses (trees 1—30) only 100 seeds of each individual sample were examined. This was owing to the object of determining seed quality quickly. Since this was in fact both easily and rapidly done by the same methods as applied in pine, 200 seeds of each sample from trees 31—50 were photographed and analysed.

By means of X-ray analysis the 50 different samples were arranged in four groups: »good seed», »medium-good seed», »less good seed» and »inferior seed», and the classification submitted to the Company. Since the empty seeds of any sample can easily be fanned away, there is no need to attach much importance to its frequency. Of major importance is the development of the full seed, belonging to embryo types I—IV.

According to Table 5, there is a striking parallelism in spruce, too, between embryo development and rate of germination. In future seed analyses this parallelism will no doubt turn out even closer, since the quality of the endosperm was not considered here. The low rate of germination in sample 18, classified as »good seed», and that of sample 19, denoted as »medium-good seed» is surprising. For a closer control the X-ray photographed samples of full seed (embryo types I—IV) in

[1] We owe our sincere thanks to these two gentlemen for their continuous interest in our studies.

TABLE 5. *Seed analysis and germination of fifty selected trees of Norway spruce.*

Tree No.	Latitude	Altitude m	Locality	Per cent of embryo type					Germination rate of full seed, per cent (STEFANSSON)
				0	I	II	III	IV	
»*Good seed*»									
1	63° 40′	90	Hörnefors	25	—	—	2	73	96
2	»	»	»	65	—	—	2	33	98
3	»	»	»	55	—	1	—	44	99
4	»	»	»	17	—	—	—	83	98
31	64° 10′	100	Olofsfors	5	—	1	—	94	100
32	»	»	»	8	—	1	—	91	98
33	»	»	»	26	1	—	—	73	98
34	»	»	»	31	—	3	1	65	98
35	»	»	»	29	1	1	1	68	96
41	63° 30′	200	Björna	27	—	1	—	72	96
42	»	»	»	33	1	—	—	66	98
43	»	»	»	12	—	1	1	86	98
44	»	»	»	25	—	—	—	75	99
45	»	»	»	8	—	1	—	91	100
36	64° 10′	200	Bäckliden	16	1	3	3	77	95
37	»	»	»	13	—	—	—	87	100
38	»	»	»	58	—	1	1	40	99
39	»	»	»	46	—	1	1	52	99
40	»	»	»	41	—	2	—	57	98
6	63° 55′	200	Bjurholm	7	1	—	1	91	97
7	»	»	»	7	—	2	1	90	100
9	»	»	»	51	1	—	1	47	100
10	»	»	»	13	—	1	2	84	98
12	63° 35′	220	Remmar-bäcken	28	—	—	2	70	99
14	»	»	»	18	—	—	—	82	99
15	»	»	»	11	—	—	1	88	99
46	63° 30′	300	Nyby	21	—	2	—	77	93
47	»	»	»	29	—	—	1	70	94
48	»	»	»	11	1	—	—	88	99
49	»	»	»	8	—	—	2	90	99
50	»	»	»	40	—	2	1	57	100
17	64° 30′	370	Vägsele	28	1	1	—	70	94
18	»	»	»	21	—	1	1	77	89
Average per cent				25,2	0,2	0,8	0,8	73,0	97,7

Tree No.	Latitude	Altitude m	Locality	Per cent of embryo type					Germination rate of full seed, per cent (STEFANSSON)
				0	I	II	III	IV	
»Medium-good seed»									
5	63° 40′	90	Hörnefors	57	2	2	1	38	95
8	63° 55′	200	Bjurholm	36	1	2	1	60	95
11	63° 35′	220	Remmar-bäcken	32	1	1	2	64	98
13	»	»	»	52	1	2	1	44	98
16	64° 30′	370	Vägsele	24	1	3	2	70	97
19	»	»	»	34	6	—	—	60	57
21	64° 10′	400	Siksjö	23	4	1	2	70	90
22	»	»	»	23	—	4	4	69	91
Average per cent				35,1	2,0	1,9	1,6	59,4	90,1
»Less good seed»									
20	64° 30′	370	Vägsele	35	5	4	2	54	69
23	64° 10′	400	Siksjö	33	7	4	5	51	96
24	»	»	»	27	5	2	3	63	96
25	»	»	»	32	2	3	3	60	92
29	65° 10′	500	Storuman	26	6	8	15	45	63
30	»	»	»	25	1	6	20	48	79
Average per cent				29,7	4,3	4,5	8,0	54,2	82,5
»Inferior seed»									
26	65° 10′	500	Storuman	6	8	61	25	—	18
27	»	»	»	25	5	19	21	30	61
28	»	»	»	32	5	15	30	18	46
Average per cent				21,0	6,0	31,7	25,3	16,0	41,7

samples 17—20 were put for germination in the Jacobsen apparatus. The results were as follows:

Sample	Full seed %				Rate of germination, within 30 days	
					STEFANSSON	Genetics Department
	I	II	III	IV	%	%
16	1,3	3,9	2,6	92,1	97,0	Not examined
17	1,4	1,4	—	97,2	94,0	97,1
18	—	1,3	1,3	97,5	89,0	96,2
20	7,7	6,2	3,1	83,1	69,0	80,0
19	9,1	—	—	90,9	57,0	74,2

Samples 17 and 18 possess higher proportions of embryo type IV than samples 19 and 20, re-examined as to germination capacity. Still, there is a certain discrepancy in the results. Whether this depends on the constitution of the endosperm or is related to unknown environmental conditions, must be left undecided at present. Distinctly correlated to the decrease in germination is, however, the proportion of embryo type I.

The table also shows that up to an altitude of at least 300 metres there is a complete germination of all seed materials, empty seed disregarded. At higher elevations, notably at an altitude of 500 metres, the seed quality declines. Samples 26—30 evidence the good correlation of embryo type and germination.

Sample	Full seed %				Rate of germination (STEFANSSON)
	I	II	III	IV	
30	1,3	8,0	26,7	64,0	79 %
29	8,1	10,8	20,3	60,8	63
27	6,7	25,3	28,0	40,0	61
28	7,4	22,1	44,1	26,5	46
26	8,5	64,9	26,6	—	18

Hence we conclude that it is possible in spruce, too, to employ X-ray photography of the embryo status as a means of predicting germination rate, quickly and satisfactorily, and to express a reliable opinion about the quality of a given seed material. As in pine the seed quality of spruce is related to the altitude of the stand, not so much with regard to the amount of empty seed but rather to the rates of embryo types I or II, which increase, and of embryo type IV, which decreases. In the four categories of Table 5 embryo type I increases from 0,2 over 2 to 4 and 6 %, embryo type II from 0,8 over 2 to 5 and 32 %. Embryo type IV, on the other hand, decreases from 73 over 59 to 54 and 16 per cent. In consequence, the measures taken by Mo and Domsjö Company cannot be argued. It was decided that cone collection should be concentrated on stands below 375 metres. In the materials examined here this procedure would lead to an average germination of 91 %, according to the mean of 40 samples, and a content of embryo type IV amounting to 95 per cent of all full seed.

Speed of germination. — For the six selected trees of Fig. 2 the average speed of germination (seeds germinated in soil within 30 days) amounts to 15, 18, 14, 18, 10, and 9 days, respectively, germination being consequently about a week retarded in the four northern trees

as compared with the two southern ones. This state of things is directly related to the quality of the seed.

The seeds examined of these trees numbered 2279. They grouped themselves in the following manner as to embryo type:

Embryo type	0	I	II	III	IV	
No. of seeds	493	204	453	330	799	= 2279
Germinated in soil, within 30 days	—	(10)[1]	59	207	750	= 1026
Per cent	—	(4,9)[1]	13,0	62,7	93,0	
Average speed of germination, days	—	—	17,9	14,8	10,6	
Germination in soil after 30 days, in % of previously germinated seeds	—	—	24,7	9,7	1,2	

[1] Misclassification.

Two features immediately show up: (1) The seeds of embryo type II germinate less quickly than those of type III and these in their turn less quickly than the seeds of type IV. (*Cf.* also the table, p. 326.) (2) The delayed germination, relatively seen, is distinctly higher in embryo type II than in type III and even more so than in type IV.

The special point of significance concerns the connection of embryo type to rate and speed of germination. This point is even better illustrated by the behaviour of graft seed. In graft seed of the four northern trees average germination was speeded up considerably: in V:2 by 6, in N:6 by 11, in Ns:6 by 5, and in Ns:8 by 7 days. Moreover, in graft seed, characterized by its exquisite degree of maturity, speed of germination was independent of seed weight, in contrast to the case in natural material. Here, in unfavourable years there is a surplus of small cones, containing an abundance of small seeds of a low embryo type, which germinate poorly and slowly. In the graft seed formed at Bogesund embryo type IV prevailed, also in small cones and in light seed, and the individual seeds germinated almost simultaneously.

Finally, the costs and labour involved in a complete X-ray analysis ought to be compared with what is valid in the common seed control practice (HUSS, 1951). Here, the manual labour with regard to 100 analyses, each comprising 4×100 seeds (*i. e.*, in all 40.000 seeds), requires 40 hours for placing the seeds in their beds of the germinator and 100 hours for the control of germination, for examination and sectioning, in all 140 hours (as a minimum value). The X-ray diagnostics of 40.000 seeds require 100 films (24×18 cm.), each with 400 seeds. The time consumed amounts to 10 hours for photography, 5 hours for development and fixation of the films and 45 hours for embryo and endosperm classification, in all 60 hours (as a maximum value). In addition, the common germination test lasts 10, respectively, 30 days per sample. In X-ray diagnostics the exposition of the sample lasts but

2—5 seconds. This comparison implies that, besides the almost immediate result, X-ray diagnostics are at most half as expensive as the ordinary method.

It ought to be mentioned here that X-ray diagnostics have been applied with success to sundry kinds of seed, of agricultural as well as wild species, for instance, *Dactylis glomerata*, *Poa pratensis*, *Alopecurus* and *Phleum* species, numerous *Plantago* species, *Rosa* types, *etc.*, in addition to other conifer species of *Pinus*, *Picea*, *Abies*, *Pseudotsuga*, and a series of hardwood species of *Betula*, *Tilia* and *Acer*.

2. Embryo type and the physiology of germination

For an understanding of the processes taking place when a seed of pine or spruce starts to germinate it is necessary to contrast the water intake of full and empty grains, in relative and absolute amounts, and to examine in an exact way the time required for the rate of germination in seeds with several small embryos or seeds with one large embryo. This latter contrast of behaviour is of special importance for a correct analysis of northern conifer seed with regard to its germinability in nature.

The material, chosen for this study, consisted of mixed seed of four pine provenances from Swedish Lapland. By means of X-ray photography all seed was classified as to embryo type (0—IV). Four samples of each embryo type, each sample consisting of 25 seeds, were used in the study. The seeds were placed on filter paper in the Jacobsen germinator and weighed individually after 1, 2, 3, 4, 5, 10, 25, 50, 75, and 100 hours. In Fig. 14 the results are given. Each point of the curve refers to the mean of four determinations.

An obvious result is the fact that empty seeds or seeds containing endosperm but no embryo absorb, relatively seen, much more water than seeds of embryo types II, III and IV, respectively. In absolute values, however, the end result is approximately identical. Independent of embryo type the seeds absorb between 2,4 and 2,8 milligrams per seed during 100 hours. This water intake must be seen in relation to the seed weight, which ranges from 1,3 to 4,8 milligrams for seeds of embryo type 0 and IV, respectively. After a time period of 100 hours seeds of embryo type IV start to germinate. A strict comparison as to the water absorption of different embryo types is thus not feasible after this time. Anyhow, the experimental results show that the existence of an embryo or, for that reason, an endosperm has no decisive effect on the processes of water absorption.

As just mentioned, germination begins at 100 hours. Germination is defined as the point where the rootlets have pierced the seed coat and reached a length of at least 5 mm. Since in this study the embryo types

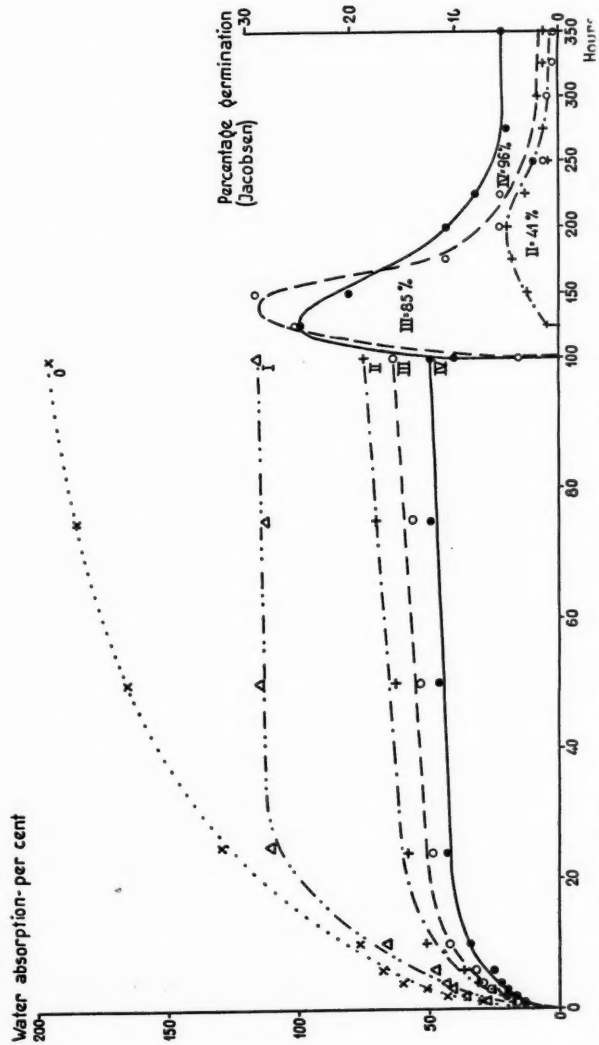


Fig. 14. The course of germination in Scots pine. Up to 100 hours only water absorption, then beginning germination of embryo types IV, III, II in the order mentioned.

have been tested separately, we obtain full information as to their reaction during germination. Embryo types III and IV show a high proportion of germinated seeds already after 100 hours, approximately twice as much in IV as in III. Embryo type IV reaches its maximum already between 120 and 130 hours. After this time germination slowly decreases and goes on to the point where almost every seed has germinated, in the actual case 96 per cent. Embryo type III gains its maximum of germination somewhat later, at 140 hours, and decreases rather rapidly below the curve of embryo type IV. The total germination is depressed, although still rather high, *viz.* 85 per cent.

With embryo type II the course of germination is distinctly changed. Here no seed has germinated before 124 hours, when type IV has already reached its maximum. After this time solitary seeds continually enter the stage of germination. Consequently, there is no pronounced peak but a very wide and flat maximum region between 150 and 250 hours. Total germination does not surpass 41 per cent for a period of 30 days. In addition, it may be mentioned that the curves of germination are markedly fixed both in embryo types IV and III. For embryo type II, on the contrary, the shape of the curve will be radically displaced by casual or intentional changes in the environment, even rather slight ones. As concluded by SIMAK and GUSTAFSSON (1954, p. 57), the great variability in germination of northern pine seed, from year to year and from locality to locality, depends on its content of embryo type II. If this is missing and III or IV abundant, the seed germinates well and rapidly. If embryo type II forms a high proportion, germination will be unsatisfactory and delayed.

3. Plant vigour and quality of seed

Since seeds of a poor developmental stage, preferably of embryo type II, germinate slowly and incompletely, in the laboratory and in outdoor sowings, the embryo status will influence the growth of the first-year seedlings, too. In fact, we can conclude from the data given by SIMAK and GUSTAFSSON (1954) that there is a positive correlation between seed weight and seedling size. This correlation primarily depends on the fact that embryo and endosperm development of one and the same genotype is further advanced in heavy than in light seeds. Graft seed of our materials often reach higher values of seedling growth than the corresponding natural seed. In general graft seed is heavier than natural seed northern in origin. In consequence, and owing to the

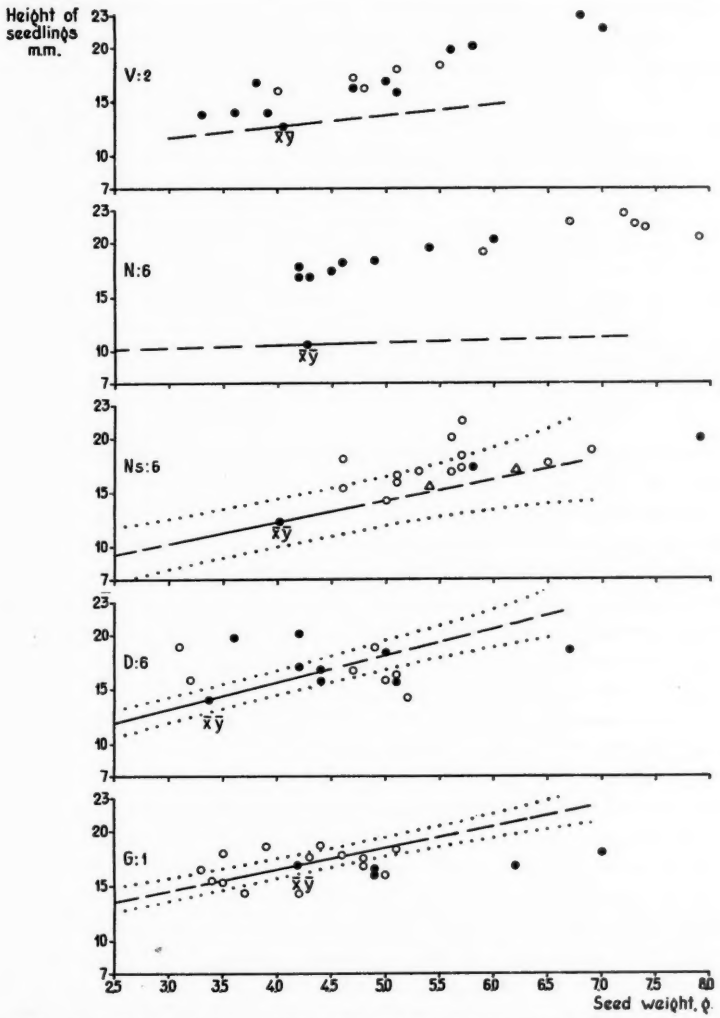


Fig. 15. The relation between 1000-grain weight and plant height in five mother trees of Scots pine (Fig. 2) and their grafts. The regression lines of the mother trees according to the equation $y=a+bx$. Triangles, as well as open and closed rings denote different grafts.

TABLE 6. *Distribution of seeds to embryo and endosperm types in eight trees of Scots pine (1000 analysed seeds per tree).*

Tree No.	Latitude	Altitude m	Per cent of seeds in embryo type							
			0	I	II		III		IV	
					A	B	A	B	A	B
21	68°	270	12,0	11,3	27,2	9,0	33,6	2,0	4,9	—
27	67° 45′	300	26,8	1,2	14,8	3,9	42,2	1,3	9,8	—
8	67° 30′	280	2,9	3,1	11,2	5,2	57,0	4,1	16,5	—
20	66° 30′	200	11,5	1,2	2,0	0,7	16,4	0,2	67,8	0,2
71	66° 16′	400	29,1	4,0	10,0	3,5	36,9	3,4	13,1	—
25	65° 10′	410	28,5	2,3	8,1	3,3	39,8	1,3	16,7	—
117	63° 40′	240	6,0	0,2	1,9	0,5	40,4	1,9	49,0	0,1
17	62° 15′	415	4,1	3,0	2,0	2,3	46,3	2,9	39,3	0,1

positive correlation between seed weight and seedling size, seedlings of graft seed will grow much better than those of natural seed. This is especially clear in genotypes like V: 2, N: 6, Ns: 6, (Ns: 8), and D: 6 (Fig. 15). Also with the seed weight kept constant the contrast between mother trees and grafts is evident, especially in N: 6 and V: 2, the two most northern trees with a surplus of embryo types II and III under natural conditions and an almost entire deficiency of embryo type IV.

Seeds of embryo type II showing a delay in germination, often postponing it several months (or even until the next vegetation period), will in consequence give rise to numerous weak first-year seedlings, which cannot survive the winter, neither under harsh natural conditions nor in the nursery.

In order to elucidate this problem more fully, eight specified trees of pine were examined as to embryo type (Table 6). Of all eight trees one hundred seeds, in four samples, were put for germination in the laboratory and their growth determined after 40 days. It is evident from Fig. 16 that the embryo type exerts a profound influence on juvenile growth. In samples 27, 21, 8, 71, where embryo type II is also represented, the order of growth is invariably IV > III > II, and in the remaining samples embryo type IV is always superior to III, except for sample 117, where they give equal results.

To a large extent the differences in growth of different samples can be brought back to conditions of the seeds, their embryo and endosperm qualities. The outstanding sample — No. 117 — has the highest grain weight, is the second southern as to origin, has a superior embryo

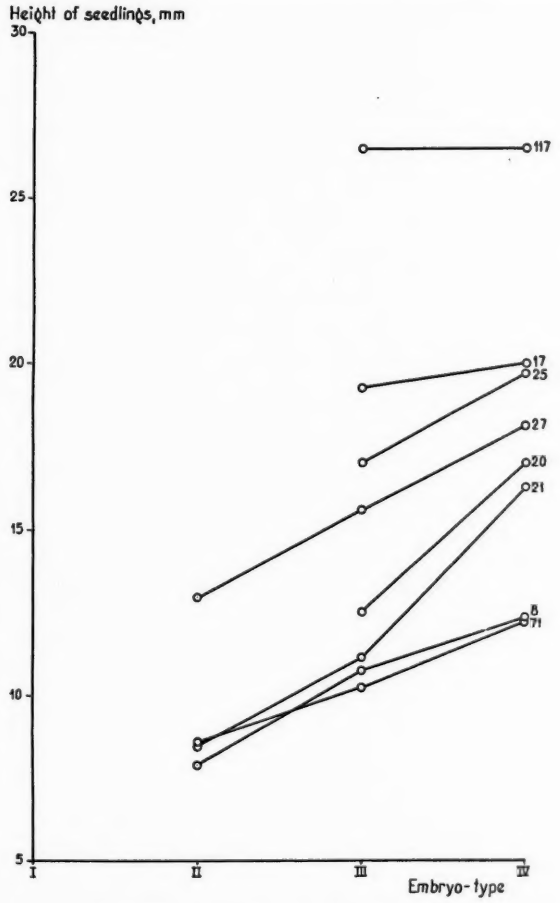


Fig. 16. The connection of embryo type and seedling growth in eight selected trees of Scots pine.

quality and an endosperm, first rate both as regards degree of development and X-ray absorption. Seed sample 17 — the second as to growth — has a low grain weight — the third lowest — but is the most southern in origin, with an excellent seed quality.

In four samples, as mentioned previously, Nos. 27, 21, 8, and 71, embryo type II was represented. As indicated by the diagram, the order

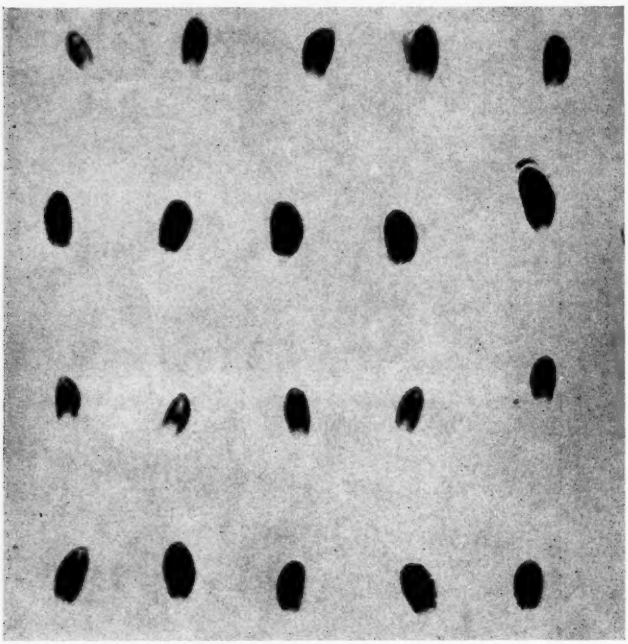


Fig. 17. Endosperm type in two northern seed samples (117 and 8), illustrating its different degree of development. First and third row: 8, second and fourth row: 117.

of growth of embryo type II is: $27 > 71 = 21 > 8$. The content of good endosperm was, respectively: 79, 74, 75, 68 %, a clear parallelism.

With regard to the growth of embryo type III the three inferior samples — 21, 8, 71 — are characterized by a partially defective or small endosperm (especially in 8 and 71), low grain weights (especially 71) and are northern in origin (sample 21 is the most northern one). The remaining samples (except for 117 and 17) are intermediate in most respects.

As mentioned above, the seed sample 17 is extraordinary in behaviour. In spite of its low grain weight it has a better growth than all the others, sample 117 excluded. The small seed is, however, most excellent as to embryo and endosperm quality partially, no doubt, caused by a favourable situation of the mother tree. This sample forms an important exception from the general rule of a correlation in grain weight and juvenile growth, but the superiority in growth is in harmony with the properties of embryos and endosperm.

In Fig. 17 we have tried to illustrate the difference as to X-ray absorption of the endosperm between samples 117 and 8. The first and the third rows depict seeds of sample 8, the second and the fourth represent sample 117. All seeds were photographed simultaneously and slightly overexposed as to emphasize specially the absorption capacity of the endosperm. The endosperm is, generally seen, much better developed in sample 117 than in 8. This holds true of all embryo types, IV as well as III and II.

Certainly, there are differences of genetical character also with regard to juvenile growth in pine. To a large extent, however, the differences within the material examined here (*Pinus silvestris* **lapponica*) can be referred to casual characteristics of the seed in relation to the climate, *viz.* in degree of embryo development, in endosperm state, in seed weight, and in maturity.

In a recent study, now under print, SIMAK (1955) has in considerable detail analysed the consequences of sifting the collected pine seed before sowing. This procedure is often considered an important means of utilizing in practice the unquestionable correlation of seed size (or seed weight), rate of germination and subsequent plant vigour. A selection by means of sifting cannot be applied adequately in mixed seed, since individual genotypes often display striking differences in grain size and weight. SIMAK examined the results of sifting in several seed samples of two provenances, one group north of the Arctic Circle (Kaupinen), the other group in South Sweden (Boxholm). Frequently the best phenotypes, approaching plus trees in appearance, possess small seeds. Consequently, they are automatically eliminated by a sifting from the, physiologically seen, most valuable seed portions. Normal or even minus trees, on the other hand, often possess a superior seed, abundant, fully mature and heavy. These trees will dominate the physiologically best seed portions. A positive selection, judged from the seed point of view, will consequently imply a negative selection for stem growth and quality.

In addition, it must be emphasized, concluding from SIMAK's study, that even in individual genotypes a hard sifting will not always lead to a seed improvement, since in natural seed the size (or weight) differences of embryo types I to IV are often inconsiderable.

4. The juvenile growth of inbred and crossbred material in pine

As evidenced in the preceding two chapters, there is a certain correlation between seed development, rate of germination and first year

TABLE 7. *Growth of 3-year-old plants obtained by different types of pollination.*

(Values within brackets are based on less than 20 plants.)

Locality	Tree No.	Year of pollination	Year of sowing	Year of measuring	Mean height (M ± m) cm.	Significance of difference 1–3 /4/ 5	Significance of difference 2–3 /4/ 5	No. of plants examined
	Open pollination							
Boxholm (1)	VIII: 1 +	1949	1951	1953	27,54 ± 0,19			1133
(2)	VIII: 2 +	»	»	»	27,56 ± 0,21			1011
(3)	VIII: 46 —	»	»	»	26,41 ± 0,24	***	***	645
(4)	VIII: 47 —	»	»	»	24,35 ± 0,05	***	***	1709
Ånge (1)	Å: 1 +	1948	»	»	21,88 ± 0,19			722
(2)	Å: 2 +	1949	»	»	27,10 ± 1,01			39
(3)	Å: 3 —	»	»	»	22,21 ± 0,11	○	***	2493
(4)	Å: 4 —	1948	»	»	20,29 ± 0,35	***	***	245
(5)	Å: 4 —	1949	»	»	[11,80 ± 1,32]	[***]	[***]	5
	Cross pollination					1–2/3/4		
Boxholm (1)	+ × +	1949	1951	1953	30,57 ± 0,30			516
(2)	+ × —	»	»	»	29,27 ± 0,39	***		298
(3)	— × +	»	»	»	28,01 ± 0,24	***		724
(4)	— × —	»	»	»	27,47 ± 0,28	***		390
Ånge (1)	+ × +	1948, 1949	»	»	22,48 ± 0,24			431
(2)	+ × —	1948	»	»	19,92 ± 0,35	***		186
(3)	— × +	»	»	»	20,10 ± 0,69	**(*)		50
(4)	— × —	1948, 1949	»	»	22,23 ± 0,19	○		922
	Self pollination					1–3		
Boxholm (1)	VIII: 1 +	1949	1951	1953	25,52 ± 1,46			21
(2)	VIII: 2 +	»	»	»	[30,5]			2
(3)	VIII: 46 —	»	»	»	18,55 ± 0,25	***		425
Ånge	Å: 3 —	»	»	»	16,39 ± 0,89			23

growth. But for how long does such a correlation hold? When do the genetic qualities set to work? These questions open up central problems for all long-time planning of forest tree breeding. ROHMEDER (1939) showed that for plants of spruce three years of age the correlation was less conspicuous than in the preceding years, although an influence of the grain weight was still noticeable. He concluded that at three years of age the hereditary constitution is quite conspicuously at work. In his paper he gave a review of earlier studies on the problem.

Since 1948 cross-pollination work has been continually and methodically carried out at the Genetics Department. In the fall of 1950 a number of inbred, cross- and open-pollinated seed material had accumulated. These were sown in 1951 and replanted as 2/0-seedlings in 1953. The plantlets were measured in October, 1953, after the end of the vegetation period (Table 7). The materials involved trees of southern and northern origin (Boxholm and Ånge).

The growth of open-pollinated materials. — The best growth of the Boxholm material occurs in the progenies of the plus trees 1 and 2. Here, the plantlets are decidedly taller than those descending from minus trees 46 and 47. The differences are significant throughout. In the Ånge material plus tree 2 has the best growth, minus tree 4 by far the lowest. Unfortunately, 2^+ is represented by no more than 39 plantlets. The plus tree 1 and the minus tree 3 lie close to one another. When lumped, the plus materials decidedly surpass the minus ones.

The growth of cross-pollinated materials. — The different combinations produced have been united into four groups: (1) plus trees×plus trees, (2) plus×minus trees, (3) the reciprocal, *i. e.*, minus×plus trees, and (4) minus×minus trees. In the Boxholm material group 1 shows the best results; it is significantly superior to the other three groups of material (P in all cases less than 0,001). The minus combinations are the inferior ones.

In the Ånge material, too, the plus-trees when crossed produce the best results. Here, however, the two extremes — plus×plus and minus×minus — do not differ significantly. On the other hand, plus×plus combinations are distinctly superior to plus×minus and minus×plus combinations ($P < 0{,}01$).

It is evident from these data that among the different intraprovenance combinations of four plus and four minus trees the plus×plus combinations are the superior ones, both with regard to open- and cross-pollination. All combinations involving the minus trees, crossed

inter se or with the plus trees, are inferior to them. How does this tally with the postulated influence of seed size on juvenile growth?

If arranged according to their growth ability, the different progenies show the following average values as to 1000 grain weight. The figures within brackets denote the number of progenies belonging to the respective growth class.

Average plant height, cm.	17,5 — 20,0	20,0 — 22,5	22,5 — 25,0	25,0 — 27,5	27,5 — 30,0	30,0 — 32,5
Average 1000grain weight, grams:						
The Boxholm material.	—	—	4,15 (2)	4,12 (1)	4,46 (5)	4,86 (2)
The Ånge material	5,11 (1)	5,33 (6)	5,51 (3)	6,35 (1)	—	—

In both types of materials the plant series which are smallest as to average height have low grain weights, and the series tallest as to growth also have high grain weights. The series intermediate as to height are also intermediate in grain weight.

However, the reported data are instructive with regard to the genetic back-ground, too. The northern materials (from Ånge) sown, nursed and replanted close to Stockholm are distinctly smaller than the southern materials (from Boxholm) handled in the same way. This is so in spite of their larger and heavier seeds. Unfortunately, the method of X-ray photography was not worked out when the seeds underlying these experiments were obtained. In consequence, we do not know the exact situation of embryo and endosperm development. However, later studies have convinced us that the inferiority as to growth of the northern materials was in this instance not associated with an inferior embryo type or a poor endosperm. Rather it is related to a retardation of growth in the northern as contrasted to the southern materials.

The importance of the genetic factors is clear from the data gathered in Table 8. Of the four southern mother trees No. 47 is in most years characterized by a rather high grain weight; in the year 1950 when the first hybrid seed of this locality was obtained, it ranked higher than plus tree 2 and almost as high as plus tree 1 and minus tree 46 as to grain weight. The progeny of this minus tree shows a stunted growth, significantly more so than the other trees. Similarly, of the Ånge trees No. 4 produces distinctly smaller plants than Nos. 1 and 3 with a very much lower grain weight. If such data as this stands a close scrutiny, when progenies of more trees have been examined, this will indicate that the growth type of many minus trees — »the minus type of

TABLE 8. *Specified growth analysis of progenies of eight trees (cf. Table 7).*

(Values within brackets are based on less than 200 seeds.)

Locality	Type of pollination	Year	Mean height (M ± m) cm.	No. of plants measured	1000 grain weight, g
Boxholm	VIII: 1 open pollination	1949	27,54 ± 0,19	1133	4,513
	VIII: 1 × VIII: 2	»	31,43 ± 0,48	251	4,942
	VIII: 1 × VIII: 46	»	31,52 ± 0,43	208	4,782
	VIII: 2 × VIII: 1	»	29,76 ± 0,37	265	4,411
	VIII: 46 × VIII: 1	»	28,01 ± 0,24	724	4,405
	VIII: 1 selfed	»	25,52 ± 1,46	21	(5,472)
	VIII: 2 open pollination	1949	27,56 ± 0,21	1011	4,251
	VIII: 2 × VIII: 1	»	29,76 ± 0,37	265	4,411
	VIII: 2 × VIII: 46	»	24,03 ± 0,55	89	(3,874)
	VIII: 1 × VIII: 2	»	31,43 ± 0,48	251	4,942
	VIII: 46 open pollination	1949	26,40 ± 0,24	645	4,623
	VIII: 46 × VIII: 1	»	28,01 ± 0,24	724	4,405
	VIII: 46 × VIII: 47	»	27,47 ± 0,28	390	4,732
	VIII: 1 × VIII: 46	»	31,52 ± 0,43	208	4,782
	VIII: 2 × VIII: 46	»	24,03 ± 0,55	89	(3,874)
	VIII: 46 selfed	»	18,46 ± 0,24	425	4,422
	VIII: 47 open pollination	1949	24,35 ± 0,05	1709	4,421
	VIII: 46 × VIII: 47	»	27,47 ± 0,28	390	4,732
Ånge	Å: 1 open pollination	1948	21,88 ± 0,19	722	4,889
	Å: 1 × Å: 2	»	23,28 ± 0,28	288	5,090
	Å: 1 × Å: 4	»	19,92 ± 0,35	186	5,106
	Å: 2 × Å: 1	1949	20,85 ± 0,41	143	5,038
	Å: 4 × Å: 1	1948	20,10 ± 0,70	50	(5,687)
	Å: 2 open pollination	1949	27,10 ± 1,01	39	(6,350)
	Å: 2 × Å: 1	»	20,85 ± 0,41	143	5,038
	Å: 3 open pollination	1949	22,21 ± 0,11	2493	5,361
	Å: 3 × Å: 4	»	24,02 ± 0,50	112	(6,216)
	Å: 4 × Å: 3	1948	22,83 ± 0,29	374	5,235
	Å: 4 × Å: 3	1949	21,26 ± 0,27	436	5,361
	Å: 3 selfed	»	16,39 ± 0,90	23	4,653
	Å: 4 open pollination	1948	20,29 ± 0,35	245	5,642
	Å: 4 × Å: 1	»	20,10 ± 0,70	50	(5,687)
	Å: 4 × Å: 3	»	22,83 ± 0,29	374	5,235
	Å: 4 × Å: 3	1949	21,26 ± 0,27	436	5,361
	Å: 1 × Å: 4	1948	19,92 ± 0,35	186	5,106
	Å: 3 × Å: 4	1949	24,02 ± 0,50	112	(6,216)

TABLE 9. *Comparison of plant heights after different types of pollination.*

Locality	Type of pollination	No. of plants measured	Mean height (M ± m) cm.	Relative values	Significance of difference 1—2/3
Boxholm	VIII: 1 self pollination (1)	21	25,52 ± 1,46	92,67	
	open pollination (2)	1133	27,54 ± 0,19	100	○
	cross pollination (3)	459	31,47 ± 0,33	114,27	***
	VIII: 2 self pollination (1)	2	(30,50)	(110,67)	
	open pollination (2)	1011	27,56 ± 0,21	100	
	cross pollination (3)	354	28,31 ± 0,34	102,74	
	VIII: 46 self pollination (1)	425	18,55 ± 0,24	70,25	
	open pollination (2)	645	26,41 ± 0,24	100	***
	cross pollination (3)	1114	27,82 ± 0,18	105,34	***
Ånge	Å: 3 self pollination (1)	23	16,39 ± 0,90	73,80	
	open pollination (2)	2493	22,21 ± 0,11	100	***
	cross pollination (3)	112	24,02 ± 0,49	108,14	***
Brunsberg	B: 70 self pollination (1)	8	33,00 ± 1,67	87,00	
	open pollination (2)	30	37,93 ± 0,86	100	**
Vägsjöfors	1 self pollination (1)	19	28,32 ± 1,75	79,21	
	open pollination (2)	12	35,75 ± 1,34	100	***
	63 self pollination (1)	56	30,23 ± 1,09	84,93	
	open pollination (2)	357	35,60 ± 0,31	100	***

growth» — is really genotypically determined and is exposed already at a juvenile stage.

Inbreeding contra outbreeding. — The X-ray analysis (p. 346) has shown that seed obtained after forced self-fertilization develop poorer embryos than open-pollinated or cross-bred material. In addition, PLYM FORSHELL (*l. c.*) provided data that open-pollination caused a more germinable seed than self-fertilization. The studies reported here evidence a similar condition as to juvenile growth.

Values are available for seven trees, five of them plus and two minus. In four trees (Table 9) there are data gathered from all three kinds of pollination, in the remaining three trees data refer to inbreeding *contra* open-pollination only.

As seen from the relative figures, plants obtained after artificial

crosses are generally superior to those obtained after open-pollination. The proportions run as 114:100***, 103:100*, 105:100***, 108:100***. There is a certain connection, here too, between grain weight and plant height. In three cases cross-fertilization has caused a greater grain weight than with open-pollination; in consequence the resulting plant height is also higher. Furthermore, within the four trees where data on cross-fertilization are gathered, there is among the different cross-combinations of any one tree an indisputable correlation of grain weight and plant height. Part of the apparent hybrid vigour can therefore be brought back to original differences in seed development and seed weight.

Forced self-fertilization leads to a marked decrease in growth. The single exception, Boxholm 2^+, cannot be considered seriously, since no more than two plants are present. The average decrease in growth, Boxholm 2^+ excluded, is estimated at slightly less than 20 per cent. The most conspicuous decrease occurs in VIII: 46^- and Å: 3^-, *viz.* 28 %. The plus trees (VIII: 2^+ excluded) show a decrease by half this figure, 14 %. The different decrease of growth after self-fertilization cannot be attributed to a difference in seed weight; no parallelism exists. Therefore, if genetic factors are at work, which seems reasonable, there must be more factors of a semilethal or viability-decreasing character present in the minus trees than in the plus trees, some of these factors being effective already in the heterozygous state, since the »minus type of growth» is a characteristic of the mother trees themselves.

Self-fertilization, on an average, results in a higher percentage of empty and poor seeds than with cross-pollination. This automatically leads to a lower number of seedlings and a decreased vigour of the plants. Seeds of minus trees certainly, according to PLYM FORSHELL (*l. c.*), produce a higher percentage of seedlings than do the plus trees. (In later, more comprehensive studies her finding was corroborated for exactly comparable materials, after selfing, open pollination as well as intraprovenance crosses. Possibly this state of things is due to the larger cones of the minus trees with the consequent automatic increase of seed size and germinability.) But the plants obtained from the plus trees are less affected by inbreeding than those from minus trees. This may be the outcome of a poorer embryo type, relatively seen, in the minus trees. The total number of seeds, as well as the percentage of germinable seeds, is higher but the seedlings are weaker owing, in addition to the »load» of viability-decreasing factors, to a poorer developmental stage of the embryos. (This in its turn may be caused by the occurrence

of semilethal factors.) That this is in fact a reality is indicated by the following comparison of plus and minus trees as to the results of self-fertilization:

Mother tree	Year	Embryo type I	II	III	IV
Boxholm 1+	1952	—	*1,7*	18,6	79,7 %
» 46−	»	*0,7*	*3,6*	10,3	85,4
Aspan 8+	»	*0,8*	*0,4*	4,1	94,7
» 17+	»	—	*3,3*	30,0	66,7
» 37−	»	—	*5,5*	27,8	66,7
» 38−	»	*1,9*	*9,3*	15,9	72,9
Boxholm 2+	1951	—	—	10	90
» 46−	»	—	—	11	89
Ockelbo 15+	»	*4*	*4*	19	73
» 18−	»	*20*	*4*	12	64
Ånge 1+	»	*14*	*24*	34	28
» 2+	»	*17*	*19*	36	28
» 3−	»	*28*	*28*	22	22

The fact seems evident that self-fertilization leads to more disastrous effects in minus trees than in plus trees, at least with regard to embryo development. As just vindicated, this seems to argue for the existence of hereditary factors, which make the minus trees develop the minus type of growth (with coarse branches and wide crowns). The minus type of growth automatically leads to a richer flowering, larger cones, increased number of seeds per cone, as well as heavier seeds per cone. In addition, when homozygosity sets in, it will result in poorer embryos in minus trees than in plus trees. Progenies of minus trees obtained after selfing, as well as after open- and cross-pollination, often show a stunted juvenile growth.

5. Seed size, juvenile growth and final production in Norway spruce (Picea Abies)

It is a common experience in Swedish forestry that continental provenances of *Picea Abies* often give a higher growth and production than native populations, as pointed out already by WIBECK (1912) and H. CARBONNIER (1937). In fact, WIBECK mentions that in the years between 1888 and 1909 no less than 35.000 kilograms of continental seed were imported and sown. A great part of the spruce stand now cultivated in the provinces of Scania and Halland may be said to originate from central European seed (LINDQUIST, 1948).

A study of juvenile growth was published by LANGLET (1941), who some years previously had begun a trial of northern and central European materials in comparison. In 1953 he reported some preliminary data of growth and quality from the international provenance tests begun in 1937. Much collection work has been carried out by KIELLANDER (1953 a, b, and previously). Recently, CH. CARBONNIER (1953, 1954) presented a searching analysis of growth, production, dimensions and capital values of South-Swedish spruce plantings, some up to 80 years of age, including also stands of central European descent.

Of special interest for this study are the data given by LANGLET (1941). He contrasted the first and second year growth of continental progenies, the seed materials of which had been directly imported (first generation) or obtained from cultures in Sweden (second generation), with that of native Swedish populations. He obtained the following values:

First year growth

Native material

Sweden: Halland. Provenance 1	$15{,}2 \pm 0{,}5$ mm
» » » 2	$14{,}4 \pm 0{,}3$ »
» » » 3	$17{,}6 \pm 0{,}4$ »

Introduced material. First generation

Germany: Pförten (lowland)	$25{,}6 \pm 0{,}6$ »
» Schwarzwald, 500 m	$24{,}8 \pm 0{,}7$ »
Poland	$26{,}3 \pm 1{,}0$ »

Introduced material. Second generation

Sweden: Halland, Sannarp	$24{,}3 \pm 0{,}4$ »
» » Tönnersjöheden	$20{,}6 \pm 0{,}4$ »

Two-year old materials

Sweden: Halland, mixed native seed	$35{,}6 \pm 1{,}1$ »
» » Sannarp, 2nd gener.	$66{,}8 \pm 1{,}9$ »

The superiority of the introduced materials as to juvenile growth is evident in all instances reported. LANGLET (*l. c.*) concludes that he has proved the hereditary back-ground of this superiority. Offhand, the case cannot be dismissed so simply. The continental spruce is often characterized by a considerable cone size and cone weight. MALMSTRÖM (1937), for instance, reports that fully developed cones of Swedish spruce reach a size in South Halland of up to 11 centimetres, those of continental spruce cultivated there up to circa 15 centimetres. The greater

size of the cones will help to increase seed size and seed weight, and by that automatically lead to a superior juvenile growth. The superiority will be even more conspicuous when using seed directly imported from southern localities and stands.

In fact, such was the case also in LANGLET's materials. The 1000 grain weight of the native Halland seed was 6,5 grams, of the Sannarp material 7,3 g and of the imported provenance seed up to 8,3 g, *i. e.*, a considerable deficit of the native seed.

A similar automatic influence on juvenile growth may be valid, too, with regard to some of the progenies studied by ANDERSSON (1947) and KIELLANDER (1953).

However, there seems to be no doubt about a partial hereditary back-ground of the superior growth, as can be seen already from WIBECK's and CARBONNIER's studies (*l. c.*, 1912, 1937). In 1953, CH. CARBONNIER showed that in the stands from south-eastern Sweden, studied by him, the foreign materials gave a much higher current volume increment than the Swedish ones, greater cubic contents, superior by some 10—20 per cent, a distinct displacement towards wider dimensions and, in consequence, a considerable gain in economical value. In his paper of 1954 he stated that of three mutually comparable plantings in Halland — called T_1, T_{19} and T_{32} — although planted at different times, the two continental ones (T_1 and T_{32}) had produced from 595 up to 718 m^3 per hectare at an age of 63 and 64 years, respectively, slightly differing with the method of thinning in the subgroups of the plantings. In the native material (T_{19}) the total production was distinctly less, from 582 up to 630 m^3 per hectare with an age of 66 years. At an age of 78 years (1951) the best T_1 plot had reached a total production of 895 m^3/hectare, the best plot of T_{32} at an age of 74 years (1952) a total production of 917 m^3/hectare. Both stands were still in active growth. The capital values of continental progenies widely surpassed those of the native ones.

The hereditary back-ground of differences in growth seems to be evident also from LANGLET's materials, 14 years of age when measured (1953). The performance of several provenance progenies was indeed remarkably striking, compared with what is true of Swedish material (Halland, Drängsered, No. 6 of Table 10), but also of provenance No. 37 (Halland, Sannarp), which represents a second generation of foreign extraction. In all 37 different progenies (Fig. 18) with up to five or six replications have been tested in three widely distant localities at Halland: Hjuleberg (56°55′ N), Bornsjön, south of Stockholm (59°15′ N) and Umeå, Bäcksjö, far towards north (63°46′ N).

TABLE 10. *The growth characteristics of provenance materials taking part in a seed orchard of spruce. (Cf. Fig. 18.)*

Provenance		All trees		10 tallest trees per plot			1000 grain weight g	No. of trees partaking in orchard		No. of grafts partaking in orchard	
No.	Origin	Average height m	Average diam. cm	Average height m	Average diam. cm	Average volume dm^3		Absolute No.	Per cent	Absolute No.	Per cent
27	Moravia, Srinosice	3,98	4,60	5,21	6,02	9,38	—	15	13,6	225	17,0
30	Roumania, Valea Bistrei	3,72	4,17	5,05	5,71	8,49	—	14	12,7	210	15,9
11	Belgium, Bullange	3,95	4,62	5,65	5,67	8,42	7,5	10	9,1	150	11,4
10	Poland, Istebna	3,82	4,38	4,97	5,68	7,98	8,9	11	10,0	110	8,3
15	Bohemia, Planice	3,90	4,32	4,98	5,57	7,72	8,5	11	10,0	110	8,3
25	Latvia, Griva	3,67	3,91	4,99	5,27	6,92	—	10	9,1	100	7,5
20	Roumania, Crucea	3,77	3,94	5,00	5,14	6,86	7,4	10	9,1	100	7,5
9	Poland, Stolpce	3,64	3,76	4,77	5,01	6,24	6,8	9	8,2	90	6,8
35	» , Radom	3,59	4,12	4,72	5,66	8,15	—	5	4,5	75	5,7
21	Roumania, Vadul Rau	3,46	3,96	4,72	5,30	6,76	9,0	5	4,5	50	3,8
37	Sweden, Sannarp, Halland, continental, 2nd generation	3,55	3,77	4,68	5,43	6,83	7,3	5	4,5	50	3,8
6	Sweden, Drängsered, Halland, native	*3,43*	*3,71*	*4,54*	*4,95*	*5,78*	*5,8*	*5*	*4,5*	*50*	*3,8*
								110	99,8	1320	99,8

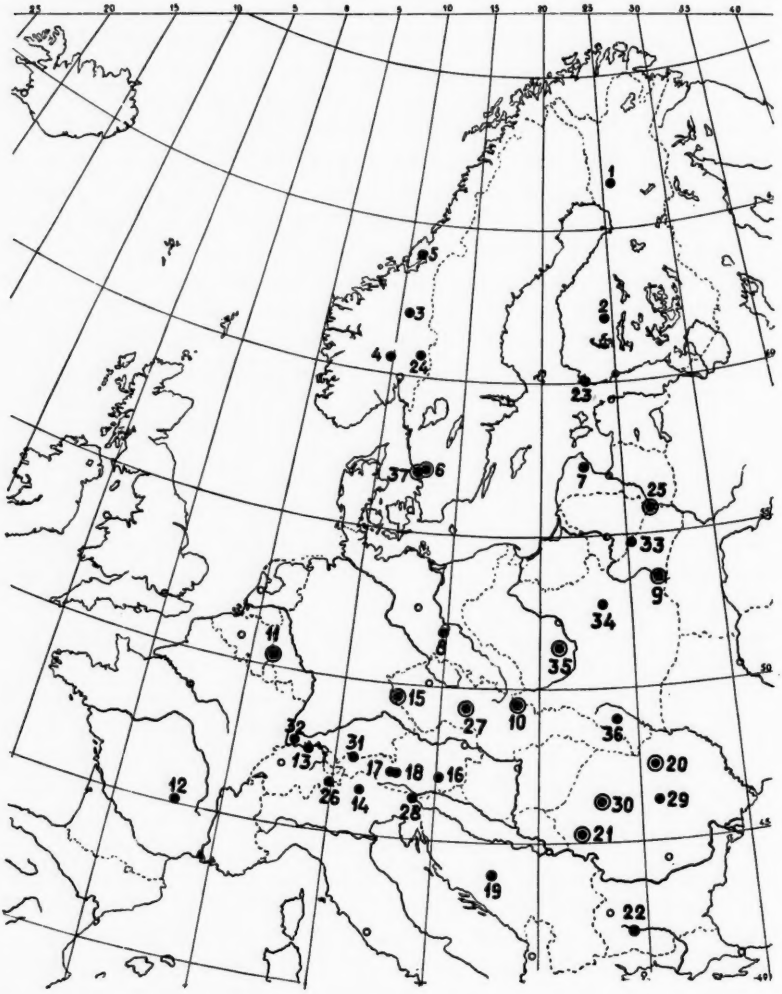


Fig. 18. Spruce provenances, tested at three localities in Sweden and now (1955) 18 years of age. Double rings denote provenances, graft materials of which take part in experimental seed orchards.

Some years ago it was thought advisable to build up special seed orchards combining experimental and practical purposes on these provenance trials, concentrating the work to the best progenies and the best performing individuals within these progenies. The superiority of the selected progenies in various characteristics at the southern station (Hjuleberg) is obvious from Table 10. Here, also the number of trees and grafts partaking in the seed orchard, devised for South-Swedish use, will be seen. The propagated treees themselves do not only surpass the mean values of all trees of the respective progenies but also those of the averaged tallest ten trees per plot. They were chiefly selected for height and diameter, but a good deal of attention was also paid to the mode of branching. In this way a population, a new ecotype, will be built up utilizing not only the superiority of the best progenies, but of their best constituents, too. Hybrid vigour will certainly be introduced into the produced seed generation. The first seed orchard of this type will be made ready in 1955 and 1956, comprising an area of circa 4 hectares. Since the seed orchard is placed in a region where cones and seed have favourable conditions of maturity (Scania: Gantofta, 56°00′ N) the seed produced will no doubt obtain well-developed embryos and endosperms, ripen well and in addition possess high 1000grain weights, owing partially to the favourable situation of the clone plantation, partially to the automatic increase of seed size in the larger and heavier cones of the foreign materials.

Table 10, moreover, shows that there is a certain parallelism between seed weight and production capacity of the foreign materials as compared with the local variety from Halland, Drängsered. The application of this parallelism ought to be a leading principle in tree-breeding: high genetic quality of seeds and plants combined with the best possible seed and plant development, in this way utilizing the physiological performance of good embryos and endosperms as well as a superior genetic constitution working into and adding its effects to the automatically high juvenile growth.

In Denmark the hereditary character of provenance determined differences in seed weight has been stressed by BORNEBUSCH (1935). He compared grain weights of original samples from 1913 with those obtained in 20-year-old plantings (1933). His data, also including plant heights in 1934, run as follows:

Origin	1000 grain weights, grams		First year growth, mm
	1913	1933	
Norway, 64° N	4,32	5,25	11,2
Finland, 61° N	5,60	7,27	18,9

Origin	1000 grain weights, grams		First year growth, mm
	1913	1933	
Latvia, 57°35′ N	6,38	7,55	19,9
Russia, 55° N	5,18	7,25	21,2
Germany, 52° N	7,80	7,80	20,7
» 51° N	7,43	8,25	22,2
» 48° N	8,48	9,06	22,3

A certain influence of the Danish climate on seed weight is noticeable. In general, however, the hereditary character of the seed weights is rather distinct. The parallelism of seed weights and first year growth can be noticed, too.

CONCLUSIONS AND DISCUSSION

The methods of X-ray photography worked out by the present authors allow a searching study of embryo development and its influence on germination and early growth. Here, we want to summarize some experimental results and discuss their consequences with regard to forest genetics and plant-breeding.

Problems of seed quality. — A fact of prime interest, in our opinion, concerns the high modifiability of embryo development in conifer seed. Unfavourable climatic conditions, whether occasional or continuous, increase the amount of empty seed and strikingly arrest the embryo development, when embryos are formed. In the conifer species studied by us, *Pinus silvestris* and *Picea Abies*, no complete adaptation of seed formation to high latitudes and altitudes occurs. Indeed, the ease with which embryos and endosperms are prevented from full development, is surprising. This fact suggests that under the course of generations the natural populations have been selected less for their good seed formation than for their vegetative properties of growth. In the case of a natural reproduction lasting over long periods this does not matter much, since two or three consecutive years of good climate, even if rare, will ensure a rich cone setting and the formation of seed of high quality. This, in its turn, will lead to a satisfactory reproduction, if the soil conditions are, on the whole, such as to allow one. Modern forestry, on the other hand, demands a rapid and complete reforestation. Here, the easy deterioration of embryo and endosperm quality implies an impeding factor.

Besides the formation of empty seed (embryo type 0) harsh exterior conditions cause seeds without embryo but with endosperm (embryo type I) or seeds with two or more small embryos (embryo type II). Also under »ordinary» climatic conditions these are of frequent occurrence in northern regions. Such seeds do not germinate, or if they germinate

do so to a scant degree. As evidenced by MÜLLER-OLSEN and SIMAK (1954), the germinability under adverse conditions of sowing — 2 cm. depth of soil — will be almost nil in the case of seed type II A, but will amount to 25 % in seed type III A and to no less than 75 % in seed type IV A. In addition, seeds of type II show a pronounced delay in germination also under favourable conditions, with a conspicuous after-germination. The delayed germination gives rise to weak first-year plants which do not easily survive the following winter season.

Grafts of northern pine trees, planted in favourable areas, produce an excellent seed material, with embryo type IV, endosperm A and high grain weights. The seeds germinate immediately, up to 95 per cent or more. This definitely proves the high modifiability of the quality characters. Several authors (SARVAS, 1950; NORDSTRÖM, 1953) stress the improving effects of visible light on dry pine seed of northern extraction with regard to its germinability. The correctness of their experiments will not be disputed here. We want to state, however, that ripe seeds of pine and spruce, if undamaged and of type IV, germinate quickly and completely. The often-discussed matter of the inferior northern seed boils down to the fact that the inferiority is quite simply a result of low embryo and endosperm quality, of types I and II prevailing instead of III and IV. The conifer seed required for reforestation in the north should be produced in sheltered clone plantations, favourably situated. Of course, this does not exclude a most intensive cone collection of plus or normal stands in years when seeds ripen well and embryos and endosperms are full-developed.

The influence of seed size on germination rate and seedling growth has been analysed in considerable detail in these and preceding studies. Under natural conditions there seems to be an even greater connection of seed size and plant height than of seed size and germination. The six mother trees repeatedly discussed (Fig. 2) show regression lines which although different with regard to inclination and level always have a positive progress. The superiority of the graft seed compared with natural seed is evident, especially so in northern genotypes. The influence of seed size on plant height is profound within one and the same type of progeny. According to ROHMEDER (1939), this influence largely disappears at an age of three years, when the hereditary differences in growth set at work instead. Also in our materials the hereditary background of growth is obvious, for instance, with regard to the properties of the Boxholm and Ånge progenies (originating from 58°10′ and 62°25′ N). The northern materials have higher grain weights than the

southern ones but the consequent growth is less, although in each group of progenies there is a conspicuous influence of seed size.

In numerous continental provenances of *Picea Abies*, when tested in Denmark and Sweden, there is a parallelism of high seed size, superior early growth and high mature production. The species stands rather wide dislodgements (LANGLET, 1948, 1952), in contrast to *Pinus silvestris*, which is split up into local populations strictly adapted to various types of soil and climate (KARSCHON, 1949). The wide adaptability of *Picea Abies* correlates, according to LANGLET (*l. c.*), to a much higher content of dry matter and reducing substances in its needles than is the case in pine. In *Picea Abies*, therefore, all properties — environmental or hereditary in character — work together and make the foreign materials outdo the native Swedish populations.

In detailed experiments we have tried to analyse the process of seed germination. An astounding fact is the enormous intake of water in empty seed of pine, approximately twice the weight of a seed. In fully developed seeds of embryo type IV the water absorption does not surpass 60 per cent. In absolute figures the intake of water is the same irrelative of the embryo type of the seed. This indicates that the water intake is a physical rather than a physiological process. The analysis also compares the time of onset of germination in embryo types II—IV and the course of the germination curves. No seeds germinated before 100 hours. Embryo type IV gained its maximum after 120—130 hours, embryo type III somewhat later, at 140 hours. In embryo type II there is no pronounced peak of germination but a flat and wide plateau between 150 and 250 hours. Seeds of embryo type II profoundly alter their mode of germination from experiment to experiment, from year to year, with even slight changes in environment. Seeds of embryo types III and IV are more locked up in their behaviour and do not change so easily. Incidentally, this contrast in behaviour explains the often capricious germination of northern seed, especially in years when embryo type II predominates.

In spite of the high modifiability of seed and embryo development there is, no doubt, a certain hereditary back-ground of seed development and seed size. In part, this is causally connected with hereditary differences as to cone size (SIMAK, 1953 b). Such a case relates to the great cone size of many continental in comparison with Swedish populations of *Picea Abies*. The greater cone size automatically gives rise to higher grain weights. However, instances are also found when large-coned biotypes have rather small seeds, and *vice versa*, although

within a genotype there always is a correlation of cone size and seed weight. Of the six pine trees, shown in Fig. 2, tree D: 6 has a remarkably small grain weight. This is true of the graft material, too. Also when cone size and seed number are kept constant, D: 6 has smaller and lighter seeds than the other five. This genotype is characterized by another singularity, too. Its amount of empty seed (embryo type 0) is conspicuously high, circa 30 per cent, both in mother trees and grafts. In most instances of graft materials, favourably situated, the empty seed will decrease in frequency (see also JOHNSSON, KIELLANDER and STEFANSSON, 1953).

Genetic properties. — An essential theme of these studies was to elucidate the influence of stock on scion properties in the case of grafting. Grafting work in Scots pine and Norway spruce often include plants of foreign provenances and species like *Pinus mugo* (*montana*) and *Picea glauca* as stock material, this especially with the object of changing onset and amount of flowering. Decisive data on this special point are not yet available. However, we have analysed the determination or, as the case may be, the flexibility of specific characters of a mother tree when grafted. It can be stated with some degree of accuracy (pp. 308—318) that the examined *characteristic* properties of a mother tree of pine, for instance, seed shape and seed details, appear in entirely identical form in mother tree and grafts. The different genotype of the roots exerts no influence whatsoever on these scion characters. Indeed, this result can be utilized in controlling the genotype of material applied in large-scale clone plantations and experiments.

On the other hand, there often appear profound differences with regard to *casual* properties like cone and seed size, number of seeds per cone, embryo type, seed germination, *etc.*, from mother tree to grafts. The characters mentioned are easily changed also in nature parallel to the variable conditions of the year. The flexibility of such characters facilitates plant-breeding considerably, with regard to its aspects of seed maturity and production.

The cone size in pine and spruce is easily influenced by changes in the environment. Grafts of pine cultivated in a favourable climate produce large and heavy cones, but the cone weight is inversely related to cone number per graft (SIMAK and GUSTAFSSON, 1954), at least when the grafts are young. In all genotypes studied (Fig. 9) the average cone weight of the grafts surpassed the values referring to the mother trees. However, there are differences which are no doubt genotypically conditioned (WETTSTEIN, 1948). Of the six trees specially examined

G: 1 has, despite its southern locality, small and light cones, more so than the more northern trees Ns: 6 and D: 6. A similar state holds true for Swedish provenances of spruce when contrasted to continental ones, also in mixed culture.

There is in our material an indisputable connection of »minus type of growth» and increased cone size. The connection was discussed by PLYM FORSHELL (1953). In all three kinds of pollination (p. 306) an analysis of variance indicates the presence of larger cones in the minus than in the plus trees. Minus trees waste much of their production on the heavy branching. In addition, they often flower more copiously than the normal or plus trees of the same stand. This implies: either that the minus type of growth actually permits a richer generative development, or that more nutrients of some special kind are available in the soil influencing in a parallel manner the type of growth and the profuse flowering. We consider that the minus type of growth is caused by specific genes, at least in some instances, since minus trees — in spite of high grain weights — often give rise to progenies, inferior in growth.

The environmental influences on seed weight are considerable, directly or indirectly, in the latter case owing to the three-dimensional connection of cone size, seed number and seed size. Consequently, minus trees should on an average produce larger seeds and better embryos. This is in fact so, in all kinds of cross-pollination. Graft individuals which ought to flower copiously and successfully in a seed orchard should be pruned in a manner giving them the minus type of appearance, even when they represent plus trees.

X-ray photography has made possible a detailed study of the effects of a forced self-fertilization. As emphasized also by PLYM FORSHELL (*l. c.*), selfing leads to a high amount of empty seed (embryo type 0), to a decrease in germination of full seed (embryo types I—IV), and to inferior progenies. The embryological picture is clear. Self-fertilization arrests embryo development. The end stage (type IV) is less frequent than in cross-pollination. Numerous embryos remain in stage I, II or III. Polyembryony (type II) is found also in trees and climates where none is seen after cross-pollination. These facts indicate that self-fertilization cannot easily be realized or, if it occurs, the zygotes are inadequately equipped genetically, often being homozygous for deleterious genes frequent in populations of conifers (EICHE, unpubl.; GUSTAFSSON, 1952).

When selfed, the examined minus trees (p. 346) produce more em-

bryos of type I and II than the corresponding plus trees of the same stand. Similarly, their selfed progenies display a more pronounced decrease in growth. This exemplifies a break in the afore-mentioned correlation of seed size and juvenile growth. It also evidences that minus trees may possess semi-recessive or even semi-dominant factors for the »minus type of growth». These factors influence the mode of embryo development in the case of selfing (*v.* above) and cause a stunted growth of the progenies.

The existence of a hereditary trend in growth properties as early as the juvenile stage and onwards is indicated by the conditions in *Picea Abies*. Here, many southern provenances are characterized by high grain weights, causing an automatic superiority in first and second year growth. This superior early growth is in many instances continued up to much greater ages. BORNEBUSCH (1935) gave an example for Danish plantings. In spite of some irregularities in their youth the employed progenies placed themselves, already a few years after planting, in an order maintained throughout from eight up to twenty years. Similar results were obtained by LANGLET (1953, and unpubl.) for 14-year-old cultures. CARBONNIER's measurements (1953) afford the conclusive evidence. The continental progenies analysed by him have for the entire period from 30 up to 70 years of age a greater annual volume growth than the native materials and, consequently, lie distinctly higher in yield. In another analysis (1954) he shows that the total yield of continental spruce may reach values of 900 m^3 per hectare or more at an age of circa 75 years, without any sign of a decrease in annual growth. Under comparable conditions native materials lie 10 to 15 per cent below in yield. The capital value of the continental progenies is even higher owing to better dimensions.

This implies that, although with great caution, we dare to correlate early growth and mature production, not for individual trees but for entire progenies. This correlation ought to be studied very carefully by physiological and anatomical means (SCHMIDT, 1951, 1953; KARSCHON, 1949), in order to find out fast methods of judging the future capacity of a progeny.

The results found in spruce show that under defined exterior conditions materials of foreign extraction may surpass the native populations as to annual volume growth and, consequently, as to yield in a defined period of time. The economical gain may be even higher than that which the crude yield figures indicate.

Projects of plant-breeding. — These considerations lead on to

problems of plant-breeding. In agriculture the primary aim of plant-breeding is to increase yield per hectare, facilitate the technique of cultivation and lower the costs of production (GUSTAFSSON and TEDIN, 1954). In forest trees the time element also enters the picture. Here the breeding program aims, briefly outlined, at new varieties, which by proper management enhance yield per time period, with the quality characters intact or improved, with a minimum of costs and labour and without an unnecessary waste of soil nutrients.

Different means of speeding up production and improving its quality have been applied both in forestry and agriculture. In modern agriculture a fertilization of the soil, especially with high nitrogen dressings, has become a most successful tool of increasing yield, not least its protein part. But, indeed, the increased fertilization puts high requirements on the varieties used. The breeder produces strains fit for high nitrogen dressings. Up to now this is scarcely true of forestry, where a manuring of the soil, however improving, is generally considered to be uneconomical, at least in the vast regions of the north.

Here, on the other hand, the plant-breeder comes across a series of far-reaching projects, among them the demand of a shortened circulation time and a greater spacing of individuals per unit planted area. The abbreviated circulation and the greater spacing require special varieties which must be produced *de novo*. Besides, many regions suffer from a shortage of soil nutrients. Here specialized varieties may be needed, too. There is in forestry, possibly more so than in agriculture, a need of breeding in converse directions, for rich and for meagre soil, for favourable and harsh localities. We are safe in concluding that hybridization and recombination, conscientiously applied, will ultimately lead to varieties adapted to the diverse types of environment. It will be possible, also in forest trees, to build up populations which work efficiently, *i. e.*, utilize minerals, nitrogen and water more thoroughly than do many old varieties.

The application of poor seed and plants in reforestation will entail a considerable economical loss in the future. Hence, it is the duty of a forester to use nothing but the best possible seed and plant material, and of the plant-breeder, on his side, to produce and release such a material.

The matter is not so easily set, however. The instruction »the best possible seed and plant material» does not only imply a genetical evaluation. A seed sample satisfactory or superior from a genetical point of view should also possess such a condition of development that

it germinates completely and quickly and gives rise to vigorous seedlings and plants. These in their turn should be raised in such a way that they do not die or stagnate when planted in the field but show a suitable growth from the outset and onwards. Finally, seed and plants perfect genetically and physiologically should be used in proper soils and climates where their superior qualities get their rights and to which they are fully adapted. A plant-breeder must, consequently, take into consideration not only genetical but also physiological and ecological aspects of forestry.

The first object in tree-breeding is thus to produce a well-developed mature seed, in conifers of embryo type IV with endosperm A. It implies for large areas a waste of time and labour to collect the poor natural seed, empty, without embryos or with several small ones (types 0—II). It scarcely germinates and produces weak seedlings. X-ray control of the seed will immediately inform one about the state of seed development. In northern regions collection of small cones is not worthwhile (Fig. 4). On the other hand, good seed years ought to be utilized to the utmost degree. The superiority of minus trees and stands with regard to flowering, cone setting and seed quality impedes the collection work. Only when no seed whatsoever is available and seed is urgently needed, collection of such trees and stands is rectified.

Clone plantations are the best means of producing a full-developed seed of embryo type IV A and of high genetic quality. The idea of planting clones of forest trees together for the production of seed was originally outlined by SYRACH LARSEN (1934, 1937), although it goes back to suggestions by FABRICIUS (1922) and OPPERMANN (1925). In Sweden, JENSEN (1943, 1945, 1954), LINDQUIST (1946) and others have proposed the establishment of numerous large clone plantations — »seed orchards». The methods of grafting are now well worked out, also for outdoor conditions (ANDERSSON and JANSSON, 1952). In future the reforestation will to a great extent rely on seed produced in clone plantations, as an addition to or, in northern countries, instead of the natural seed. The genetic quality of the seed, as well as its physiological state, will continually become improved. In *Pinus silvestris* the seed orchards will be built up for the production of seed applicable within rather narrow limits, owing to the specialized needs of the local populations of this species. In *Picea Abies*, on the contrary, the seed materials may be fit for use in much larger areas.

There are different systems possible in establishing a clone plantation (GUSTAFSSON, 1952). Conifer trees, which are apt to self-fertilization,

and this is not too rare an event (PLYM FORSHELL, 1953), should be avoided in large-scale grafting work, since their progenies will often decrease in yield. Similarly, it is a demand from the genetic point of view that the plantations should be protected from pollination by surrounding stands or trees of the same species. Indeed, the danger of such a contamination is profound (ANDERSSON, 1955).

(1) *Seed orchards producing common or »stand seed»*: The clones partaking do not always represent approved plus trees, especially is this the case for exposed districts where they cannot regularly be found. Or, if the partaking trees are of an approved plus character, there is in the orchard a contamination with foreign pollen. This more primitive type of seed orchard may fill an acute demand of conifer seed and will no doubt (p. 321) be of significance for the formation of high-altitude populations, previously not existing or but poorly adapted to the harsh conditions, *i. e.*, new ecotypes. X-ray photography, now begun on a large scale, has indicated the existence of single genotypes adapted to these conditions also with regard to seed formation.

(2) *Seed orchards built up on approved plus trees* (or on so-called élite trees, if their progenies have been found to surpass a chosen standard material): This orchard type rests on a careful selection of phenotype-controlled individuals (p. 308) and their systematic recrossing. Owing to its establishment on the basis of genotypes originating from a restricted region it is here called the *intraprovenance* type of clone plantation.

(3) *Seed orchards utilizing the phenomenon of hybrid vigour*: Heterosis or hybrid vigour is of frequent occurrence also in forest trees, either realized by the crossing of different provenances within a species or of different species with one another. In conifers it has been described by DENGLER (1932, 1939) and RIGHTER (see, for instance, STOCKWELL and RIGHTER, 1947; RIGHTER and STOCKWELL, 1949). The orchards are *interprovenance* or *species hybrid* in character. In spruce the intercrossing of high-productive geographically isolated populations may produce fundamental improvements, replacing native materials. In pine the intercrossing of different species, for instance, *P. Murrayana* and *Banksiana*, is said to raise growth considerably, combine good quality and high production and in some instances give rise to combinations resistant to diseases. Finally, this type of orchard may be established on the basis of *inbred lines*. More and more progenies of selfed pine and spruce will become available in forest-genetical work. The intercrossing of inbred lines will possibly in some instances effect a higher heterosis

than in the other types of heterotic orchards. Here also the production of inbred lines *via* induced haploidy, as successfully done by CHASE (1953) in corn, may be considered.

By the scheduled means forestry will successively acquire breeding materials, in which physiological and genetical factors are combined: a perfect physiological state of the seed with its consequent gain in juvenile growth, and then the improving effects of inherent qualities. Circulation time will be shortened by using fast-growing progenies of the hybrid type. Most probably a greater spacing of the plantings will be made possible if the seed used is based on the intercrossing of high-quality plus trees, with a light branching even when growing freely, in this way decreasing costs and labour in planting and still guaranteeing stand quality. Finally, total production will be raised in a defined period of time, if the populations used are adapted to the specific types of soil and climate in a precise manner. So far, casual events of seed migration and reforestation have more or less counteracted the establishment of fully adapted, high-productive populations. That the historical realization or selection of a local population does not necessarily lead to the best results from man's point of view, is visualized for Sweden's part by the superior performance of numerous foreign material in *Picea*.

SUMMARY

(1) X-ray photography makes possible a detail analysis of embryo and endosperm development in conifers (*Pinus* and *Picea*) as well as deciduous trees (*Alnus*).

(2) Seed development is highly influenced by environmental conditions. At high latitudes and altitudes the embryo and endosperm quality deteriorates considerably. The conspicuous modifiability is shown by the formation of poor seed in northern mother trees of pine compared with a distinct improvement in graft materials cultivated further south.

(3) Self-fertilization leads to a break-down and arrest of embryo development, often resulting in a high amount of empty and polyembryonic seed. A similar result is obtained in species crossings.

(4) In general so-called minus trees produce more and larger cones than plus and normal trees of the same stand. Parallel to this increase in cone size there is a rise in grain weight, owing to a three-dimensional connection of cone size, seed number and seed size.

(5) An analysis of seed formation in pine trees of an Arctic population indicates the presence of genetically determined differences in embryo development under harsh exterior conditions. The amount of empty seed is a highly modifiable property but is in some instances characteristic of individual genotypes, like, for instance, D: 6 (Fig. 3). New ecotypes may be synthesized by intercrossing genotypes selected not only for their growth properties but also for their superior seed quality.

(6) Also in *Alnus* polyembryony was discovered by means of X-ray photography (Fig. 12). There seems to be a genetical back-ground of this property.

(7) Seed properties are compared in mother trees and grafts of pine. The *characteristic* properties, like seed shape and seed details, are remarkably constant within individual genotypes. *Casual* properties, on the other hand, like seed size and seed weight, change considerably from mother tree to grafts and from year to year. The constancy of the characteristic seed properties can be used in breeding work as a means of genotype control.

(8) Embryo and endosperm quality greatly influence germinability and seedling growth. X-ray photography, giving an exact picture of the seed interior, may partially replace the more expensive, circumstantial and time-consuming ordinary methods of seed control. Also seed damage, caused by careless extraction and dewinging, as well as insect attacks, are readily discernible.

(9) The course and extent of germination were analysed in special experiments. The intake of water in germination appears to be physical rather than physiological in character, since it is, relatively seen, identical in empty, polyembryonic and fully developed seeds, leading to the same absolute amounts of absorbed water after 100 hours, when germination becomes visible.

(10) Growth characteristics after open-, cross- and self-pollination are compared for progenies of plus and minus trees of pine. Although the increase in cone size of minus trees will cause a higher grain weight and a consequent increase in juvenile growth, progenies of minus trees are often, even at the age of three years, characterized by stunted growth. This and other properties suggest the existence of semi-dominant (or semi-recessive) factors related to the minus type of growth, in its qualitative and quantitative aspects. The importance of genetical factors is further emphasized by the comparison of northern and southern progenies in *Pinus*.

(11) Growth properties of *Picea Abies* are analysed in some detail

with regard to the superiority of foreign provenance material to native Swedish populations. A connection exists with regard to large cone size, high grain weights, the automatic increase of seedling growth and a genetically determined superior production.

(12) In the concluding chapter some problems of seed quality, plant vigour and production capacity are discussed from genetical and environmental points of view. Different types of clone plantations are briefly outlined. The progress and aims of plant-breeding are compared for forestry and agriculture.

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STUDIES OF OPPOSITIONAL ALLELISM IN TRIFOLIUM NIGRESCENS

By *JAMES L. BREWBAKER*
INSTITUTE OF GENETICS, UNIVERSITY OF LUND, SWEDEN

AMONG the clover species, *Trifolium nigrescens* VIV. is an annual species with 2N=16 which has been suggested as one of the ancestors of 32-chromosome *T. repens* L. (BREWBAKER and KEIM, 1953). It occurs naturally in restricted areas of countries north of the Mediterranean Sea, and is cultivated sparingly as a pasture legume in the southern United States. All the diploid plants of *Trifolium nigrescens* used in this study were self-incompatible. The primary object of the investigation was to determine the genetic mechanism conditioning this self-incompatibility.

Seed lots were obtained from the Bureau of Plant Industry of the U. S. Department of Agriculture and from the Swedish Seed Association, Svalöf. All plants were grown to maturity and manipulated for pollination in the greenhouse. Heads were trimmed to ten suction-emasculated florets for most self- and cross-pollinations. In case more than a single cross was made for any one combination, the maximum seed set was recorded in the tables to be presented.

EXPERIMENTAL RESULTS

No seeds were obtained when several heads on each of ten diploid plants (designated n1, n2 . . . n10) of *T. nigrescens* were self-pollinated. All of the 68 intercrosses (ten florets per cross) made among these ten plants were fertile, averaging 11,4 seed per cross.

Ten F_1 plants of the cross n1×n2 were selfed and diallelly intercrossed (Table 1). Backcrosses of these F_1 plants to their parents could not be made since parental clones died after the first flowering season. No seed was obtained from self-pollinations of the ten F_1 plants, and selfing data were consequently omitted from Table 1. Cross-incompatibilities revealed three intra-sterile, inter-fertile groups (designated A, B, and C) with 1, 6, and 3 plants, respectively. The average seed set of the 54 compatible crosses was 10,9, and differences among seed set averages of the ten plants as males and as females were non-significant.

TABLE 1. *Seeds obtained when 10 F_1 plants of the cross n1×n2 were diallelly intercrossed.*

Plants used as female	Seeds per 10 florets from crosses with following plants as males										Av. comp. crosses
	A	B						C			
	1	2	3	5	7	8	9	4	6	10	
A— 1	—	9	11	4	1	15	4	10	3	7	7,1
2	12	—	0	0	0	0	0	11	12	4	9,8
3	14	0	—	0	0	0	0	9	13	17	13,2
B— 5	9	0	0	—	0	0	0	15	10	11	11,2
7	12	0	0	0	—	0	0	16	6	10	11,0
8	9	0	0	0	0	—	0	16	15	12	13,0
9	17	0	0	0	0	0	—	10	10	8	11,2
4	16	21	11	18	14	14	12	—	0	0	15,1
C— 6	16	16	14	13	5	8	14	0	—	0	12,3
10	2	4	15	15	7	5	7	0	0	—	7,9
Av. comp. crosses	11,9	12,5	12,8	12,5	6,8	10,5	9,3	12,4	9,8	9,8	10,9

Of the six intercrosses among the three F_1 groups, the following three were grown for progeny classification.

(1) *B×A.* — Progeny from crosses of five group B plants as females with the group A plant as male were studied. The fertility relationships of preliminary intercrosses suggested the occurrence of two intra-sterile, inter-fertile groups among the 18 plants. One representative plant of each of the two groups was used as female tester in crosses to the remaining 16 plants as males (Table 2). Each of the F_2 plants was cross-incompatible with one of the testers and cross-compatible with the other. The two intra-sterile, inter-fertile groups comprised 11 and 7 plants, respectively.

(2) *C×A.* — Progeny from two crosses of group C plants as females with the group A plants segregated into four intra-sterile, inter-fertile groups in preliminary intercrosses. One representative plant of each of the four groups was used as female tester in crosses to the remaining

TABLE 2. *Seeds obtained when 18 F_2 plants of the F_1 cross B×A (Table 1) were crossed as males to 2 tester F_2 plants.*

Plants used as female	Seeds per 10 florets from crosses with following B × A plants as males																	
	1	2	4	5	9	11	14	15	16	22	23	3	7	8	10	13	19	20
B×A—5	0	0	0	—	0	0	0	0	0	0	0	6	5	2	9	8	5	10
B×A—7	3	5	5	4	5	3	4	2	7	2	3	0	—	0	0	0	0	0

TABLE 3. *Seeds obtained when 19 F_2 plants of the F_1 cross C×A (Table 1) were crossed as males to four tester F_2 plants.*

Plants used as female	Seeds per 10 florets from crosses with following C × A plants as males																			
	2	11	18	20	23	4	7	8	9	10	16	17	19	5	14	1	6	13	22
C×A— 2	—	0	0	0	0	15	10	5	10	8	9	13	4	9	9	10	11	3	7
C×A—10	10	7	8	12	7	0	1	0	0	—	0	0	0	3	4	8	9	2	8
C×A—14	17	16	2	8	11	10	8	12	10	7	13	11	3	0	—	6	4	2	7
C×A—22	14	13	16	10	11	8	6	4	4	3	8	8	12	4	7	0	0	0	—

plants as males (Table 3). The four groups comprised 5, 8, 2, and 4 plants, respectively.

(3) *C×B.* — Progeny from five crosses of group C plants as females with group B plants segregated into two intra-sterile, inter-fertile groups in preliminary intercrosses. One representative plant of each of the two groups was used as female tester in crosses (Table 4). The two groups comprised 7 and 11 plants, respectively.

The results of this series of crosses can be explained by assuming that the self- and cross-incompatibilities are conditioned by oppositional *S* alleles of the gametophytic type. Parent plants n1 and n2 are suggested to have differed in both alleles, *i. e.*, they may be designated S_1S_3 and S_3S_4, respectively. The single plants of F_1 group A was arbitrarily assigned the genotype S_1S_3. The segregation of two intra-sterile, inter-fertile groups in the B×A progeny (Table 2) showed that B and A had one *S* allele in common, and group B was arbitrarily designated S_2S_3. The segregations of four groups in the C×A progeny (Table 3) and of two groups in the C×B progeny (Table 4) indicated that group C represented the genotypic class S_2S_4. The absence of the S_1S_4 genotype among the F_1 plants of n1×n2 is considered to be a consequence of inadequate sampling of this progeny. The expected genotypic segregations of the three F_2 progenies are given below:

$$\text{B}\times\text{A}\quad S_2S_3\times S_1S_3 \rightarrow 1\ S_1S_2+1\ S_1S_3$$
$$\text{C}\times\text{A}\quad S_2S_4\times S_1S_3 \rightarrow 1\ S_1S_2+1\ S_1S_4+1\ S_2S_3+1\ S_3S_4$$
$$\text{C}\times\text{B}''\quad S_2S_4\times S_2S_3 \rightarrow 1\ S_2S_3+1\ S_3S_4$$

TABLE 4. *Seeds obtained when 18 F_2 plants of the F_1 cross C×B (Table 1) were crossed as males to two tester F_2 plants.*

Plants used as female	Seeds per 10 florets from crosses with following C × B plants as males																			
	3	7	9	10	11	20	23	1	2	4	6	14	16	18	21	24	27	28		
C×B—23	0	0	0	1	0	1	—	19	14	13	20	2	2	8	4	12	8	8		
C×B—24	8	17	9	8	10	13	10	0	0	0	1	0	0	0	0	—	0	0		

TABLE 5. *Seeds obtained from intercrossing among tester F_2 plants.*

Plants used as female	Seeds per 10 florets from crosses with following tester plants as males							
	B×A —5	C×A —14	C×A —10	C×B —24	B×A —7	C×A —22	C×B —23	C×A —2
B×A— 5	—	0	12	3	5	14	7	10
C×A—14	0	—	7	5	5	7	4	17
C×A—10	6	4	—	0	5	8	6	10
C×B—24	5	4	0	—	2	5	10	3
B×A— 7	4	3	2	3	—	2	3	2
C×A—22	10	7	3	6	6	—	0	14
C×B—23	2	4	4	12	8	0	—	11
C×A— 2	14	9	8	14	4	7	8	—

The observed F_2 segregations conformed statistically to the expected ratios. The eight tester F_2 plants from the three progenies were intercrossed, revealing five intra-sterile, inter-fertile groups (Table 5). These are considered to represent the five different genotypes expected in the three progenies. The S_2S_4 genotype (as F_1 group C) was not recovered among the F_2 plants.

The hybrid progeny of n2 and an unrelated clone n3 were similarly intercrossed to determine their incompatibility relationships (Table 6). No seed was obtained when the parents and 22 F_1 plants were self-pollinated, and these data were omitted from the table. Fifteen of the F_1 plants were used as pollen parents for F_1 intercrosses. The 22 plants segregated into four intra-sterile, inter-fertile groups of 8, 7, 4 and 3 plants, respectively. Parental plants n2 and n3 thus were considered to have differed in both *S* alleles. Among the progeny of such a cross, four genotypes are expected in equal numbers, for which ratio the observed segregation is statistically satisfactory (Chi-square = 3,091, P = 0,50—0,30).

The 237 compatible crosses among F_1 progeny of n2×n3 (Table 6) averaged 6,2 seed per ten florets. The data from compatible crosses were first analysed independently for females and males on a between-within basis for genotypes, plants within genotypes, and crosses within plants (Table 7). No significant differences were observed among the genotype averages. The seed set averages of the 22 plants as females ranged from 2,0 to 11,4. These differences were highly significant, indicating heritable differences in seed setting irrespective of *S* genotype.

The seed sets of compatible crosses of n2×n3 progeny were also analysed separately for each of the four F_1 groups, utilizing female

TABLE 6. *Seeds obtained from intercrosses of 22 F_1 plants of the cross n2×n3.*

Plants used as female		Seeds per 10 florets from crosses with following plants as males															Av. comp. crosses
		A					B					C			D		
		2	3	8	14	21	9	12	15	18	19	7	10	16	4	22	
	2	—	0	0	0	0	5	12	5	3	11	13	12	9	3	6	7,9
	3	0	—	0	1	0	9	5	5	4	3	4	2	3	8	2	4,5
	6	0	0	0	0	0	5	8	14	14	12	18	9	13	11	10	11,4
A—	8	0	0	—	0	0	6	2	4	8	6	5	6	5	10	3	5,5
	13	0	0	0	0	0	7	6	10	8	12	5	5	7	7	11	7,8
	14	0	0	0	—	0	3	4	5	4	1	8	4	4	2	4	3,9
	20	0	0	0	0	0	6	10	12	13	12	12	2	6	6	3	8,2
	21	0	0	0	0	—	6	4	6	14	4	11	7	3	9	13	7,7
	1	6	6	3	11	7	0	0	0	0	0	7	10	6	6	3	6,5
	9	5	2	6	8	5	—	0	0	0	0	3	13	9	3	10	6,4
	11	2	2	2	2	1	0	0	0	0	0	4	2	1	2	2	2,0
B—	12	8	15	6	11	6	0	—	0	0	0	14	3	5	11	8	8,7
	15	7	6	11	4	5	0	0	—	0	0	4	4	4	2	6	5,3
	18	3	17	2	11	5	0	0	0	—	0	18	12	16	10	8	10,2
	19	15	9	3	10	5	0	0	0	0	—	3	6	9	4	6	7,0
	7	7	2	7	5	2	9	9	6	8	6	—	0	0	6	9	6,3
C—	10	6	0	1	0	3	4	8	2	5	2	0	—	0	0	1	2,7
	16	7	2	3	4	7	3	4	2	4	6	0	0	—	3	7	4,3
	17	9	3	2	4	6	2	3	9	5	1	0	0	0	5	2	4,2
	4	8	8	4	10	13	13	6	7	12	8	14	10	6	—	0	9,2
D—	5	8	2	2	3	7	3	2	2	4	4	7	2	4	0	0	3,8
	22	4	5	2	2	7	3	4	3	7	4	2	7	4	0	—	4,2
Av. comp. crosses		6,8	4,6	2,9	5,1	4,6	5,6	5,8	6,1	7,5	6,1	8,4	6,4	6,3	5,7	6,0	6.2

TABLE 7. *Variance analyses for seed sets of compatible crosses among n2×n3 progeny (Table 6).*

Source of variance	*D. F.* Mean Square for analyses of data with plants used as	
	Females	Males
Genotypes	*3*/80,74	*3*/23,77
Plants	*21*/71,72**	*14*/15,92
Error	*212*/ 7,62	*219*/14,01

** Exceeds F for P=0,01.

TABLE 8. *Female plant×male group variance analyses of seed sets in Table 6.*

Analysis of	Source of variance: Female plants D. F.	Female plants M. S.	Male groups D. F.	Male groups M. S.	Female plants × Male groups D. F.	Female plants × Male groups M. S.	Error D. F.	Error M. S.
Group A	7	58,21*	2	1,32	14	14,70	56	9,29
» B	6	67,38*	2	9,78	12	14,42	49	12,29
» C	3	27,08	2	4,40	6	7,66	36	5,65
» D	2	115,41**	2	1,64	4	1,62	30	6,84

* Exceeds F for P=0,05.
** » » » » =0,01.

plant×male group interactions as error (Table 8). Significant differences were indicated among plants as females within F_1 groups A, B, and D. Female plant×male group interactions were non-significant, indicating that the plants as females did not respond differentially in their seed sets to the different genotypic groups as males. The differences among genotypes as males, as well as among plants as males within the genotypes, were non-significant (Tables 7 and 8). It is evident that the *S* genotype of the male parent did not significantly affect seed sets in this family of *T. nigrescens*. Similar results have been obtained with diploid material of *T. repens* and *T. hybridum,* while the genotypes of male parents in crosses of autotetraploid *T. repens* significantly affected seed sets (*cf.* ATWOOD and BREWBAKER, 1953).

Colchicine applications resulted in the production of ten autotetraploids of *T. nigrescens*. Six of these plants, including c-doubled sectors on plants n1 and n2, were self-pollinated. No selfed seed was obtained on any of the plants. Open-pollinated seed of the six 4N plants was bulked for seed increase. Of the 27 4N plants in the second generation which were tested for selfing behaviour (five entire heads per plant), ten were classified as self-compatible. Plant averages of the ten self-compatible plants ranged from 2,3 to 14,3 selfed seed per head, with an overall average of 6,9 seed per head. In contrast, seed sets of the 17 plants classified as self-incompatible ranged from 0,0 to 1,0, with an overall average of 0,1 selfed seed per head. It may be inferred that self-compatibility in autotetraploid *T. nigrescens* is a result of the functioning of the competition type of heterogenic *S* allele pollen combinations, similar to those combinations which have been described in *T. hybridum* (BREWBAKER, 1953) and *T. repens* (BREWBAKER, 1954).

DISCUSSION

Self- and cross-incompatibilities are widely dispersed among flowering plants, and are conditioned by several different types of genetic mechanisms (see BATEMAN, 1952). The incompatibility relationships in *T. nigrescens* conform to the type which was first presented with supporting data for *Nicotiana* spp. in which a series of multiple oppositional alleles is operative. Pollen reaction is of the gametophytic type, *i. e.*, it is under the control of the allele in the pollen. LEWIS (1949) listed a group of species in which self- and cross-incompatibilities

TABLE 9. *Homomorphic species in which incompatibilities are under control of a single series of multiple oppositional alleles.*

Species	Gametic Number	Family
I. GAMETOPHYTIC CONTROL (»*S*« alleles)		
Nicotiana spp.[1]	9	Solanaceae
Petunia axillaris	7	»
» *integrifolia*	7	»
Solanum spp.	12	»
Antirrhinum glutinosum	7	Scrophulariaceae
Nemesia strumosa	9	»
Veronica syriaca	7	»
Oenothera organensis	7	Onagraceae
» *rhombipetala*	7	»
Papaver nudicaule	7	Papaveraceae
Prunus avium	8	Rosaceae
Tradescantia virginiana	12	Commelinaceae
Trifolium hybridum	8	Leguminosae
» *nigrescens*	8	»
» *pratense*	7	»
» *repens*	16	»
II. SPOROPHYTIC CONTROL (»*R*« alleles)		
Cosmos bipinnatus	12	Compositae
Crepis capillaris	3	»
» *foetida rhoedifolia*	5	»
Parthenium argentatum	18	»
Capsella grandiflora	8	Cruciferae
Iberis amara	7, 8	»
Theobroma cacao	8	Sterculiaceae

[1] *Nicotiana alata*×*N. forgetiana.*

were under the control of oppositional *S* alleles of the gametophytic type. In recent studies the following species have been added to this group: *Petunia axillaris* and *P. integrifolia* (STOUT, 1952), *Trifolium hybridum* (WILLIAMS, 1951; BREWBAKER, 1951), and *T. nigrescens*. LAMM (1952) has interpreted data from preliminary studies of *Lycopersicon peruvianum* MILL. on the *S* allele scheme. In addition, a considerable body of evidence from several studies appears to support the inclusion of *Pyrus communis* in this group. The *S* allele system has been reported in species belonging to seven different plant families (Table 9). BATEMAN (1952) considers that such widely dispersed occurrence is indicative that multiple allelic incompatibility has arisen *de novo* many times in the evolution of angiosperms.

Results from incompatibility investigations in *Crepis foetida rhoedifolia* and *Parthenium argentatum* were interpreted by HUGHES and BABCOCK (1950) and GERSTEL (1950), respectively, on the basis of oppositional *R* alleles of the sporophytic type, in which pollen behaviour was determined maternally. Unlike the gametophytic *S* alleles, *R* alleles exhibit dominance in both the pollen (superimposed maternal effect) and the style. Species in which the incompatibility system has been suggested to be under the control of sporophytic *R* alleles include *Cosmos bipinnatus* (GROWE, 1954), *Crepis capillaris* (BREWBAKER, unpublished), *Iberia amara* (BATEMAN, 1954) and *Theobroma cacao* (R. L. KNIGHT, unpublished). In addition, BATEMAN (personal communication) has re-interpreted RILEY's data (1936) on *Capsella grandiflora* on the basis of the operation of *R* alleles. A summarized list of species with multiple oppositional allelic incompatibility system appears in Table 9.

SUMMARY

All plants of 2N *Trifolium nigrescens* studied were found to produce little or no seed upon self-pollination. Self- and cross-incompatibilities were studied in several F_1 and F_2 families, and were shown to be under control of oppositional *S* alleles of the gametophytic type.

Self-compatible autotetraploids were obtained in an F_2 population derived from self-incompatible c-doubled clones. It was suggested that this self-compatibility occurred as a result of the competition type of interaction of *S* alleles in heterogenic pollen grains produced by these plants.

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Appendix. — Since this article was submitted, LUNDQVIST has published results of his incompatibility researches in *Secale cereale* (LUNDQVIST, A. 1954. Studies on self-sterility in rye, *Secale cereale* L. — Hereditas 40: 278—294). LUNDQVIST reports that incompatibilities in rye may be explained best by the operation of duplicate series of oppositional S alleles, independently inherited. Such a system also may be operative in *Beta vulgaris*.

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SOME PRELIMINARY OBSERVATIONS ON CROSSING OVER AND NON-DISJUNCTION

By *RAPHAEL FALK*

INSTITUTE OF GENETICS, UNIVERSITY OF STOCKHOLM

I. CROSSING OVER IN MATROCLINOUS DAUGHTERS OF XXY FEMALES

WHEN BRIDGES (1916) was doing his experiments with XXY females he could recognize their constitution only through a progeny test. He presented however the progeny of nearly a hundred XXY females, showing that the two X chromosomes were non-crossovers. This fitted his theory that these females are produced from eggs in which one X chromosome was introduced after pairing with the Y chromosome, and the other through random movement, as it did not pair at all in meiosis. But on studying triploid females BRIDGES and ANDERSON (1925) found that the X chromosome which did not pair proximally, and therefore moved randomly to one of the poles, can pair and exchange parts distally. A crossing over picture was obtained in which the frequency of homozygosis in matroclinous daughters of the triploids increased toward the distal end of the X chromosome. If the matroclinous daughters of XXY females are really produced through heterologous pairing of the X and Y chromosomes at their proximal end it would be surprising if the two Xs did not pair distally. This holds especially since the Y chromosome has a part homologous to the X only proximally in the heterochromatin, and since pairing in every region always involves two of the three chromatids in triploid females. According to STURTEVANT and BEADLE (1936), there have been found a few cases of cross over strands in the matroclinous daughters of the XXY females, but with such a low frequency that they can be neglected in practically all calculations.

With the aid of the sc^s.Y chromosome (MULLER, 1948) it is possible easily to recognize the presence of XXY females by marking both Xs with yellow and using the sc^s.Y chromosome, which includes the normal allele of yellow. Thus it is relatively easy to get a great number of marked XXY females and to analyse their matroclinous daughters. The necessary stock was synthesized from a y ec ct v f/y ec ct v f/sc^s.Y stock in the manner shown in Fig. 1. The M5 *ry* chromosome is a

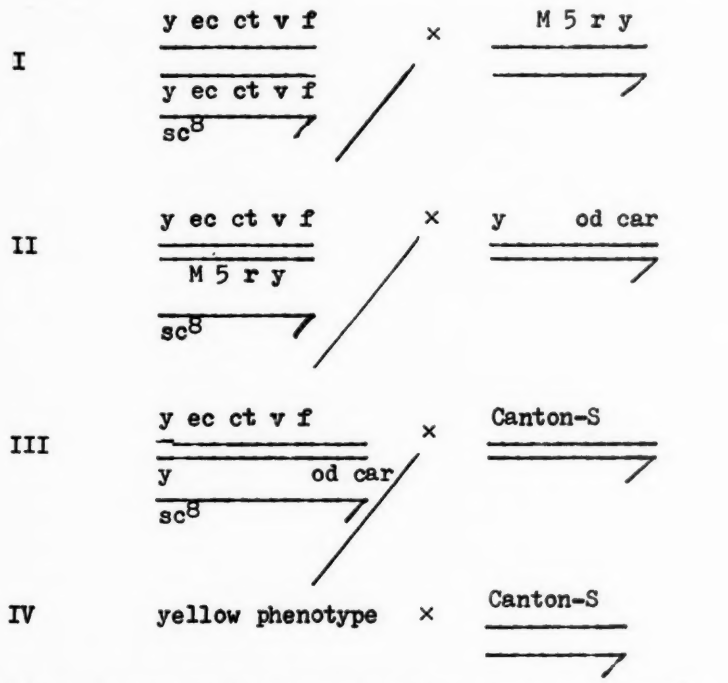

Fig. 1. Scheme of crosses done for analysing the genotype of matroclinous daughters of XXY females.

Muller-5 chromosome which has round eyes and yellow body colour. As can be seen, all yellow females in line IV of Fig. 1 are matroclinous daughters of XXY females. If crossing over happens between the two maternal Xs, it is expected to result in a quarter of the cases being homozygous with regard to the markers to the left of the point of crossing over. Among some 250 such yellow females none showed homozygosis for any of the markers (Fig. 2). 113 of these females have been crossed individually to Canton-S males and their sons classified, in order to identify the exact constitution of the X chromosome. Only one female could be classified as having cross over chromosomes; it was of the genotype y ec ct od car/y v f/Y. It is possible that this female is not the result of a regular meiotic exchange. This experiment thus fully confirms the previous experiments of BRIDGES and others, and appears not to go in line with BRIDGES' theory of non-disjunction, but fits better

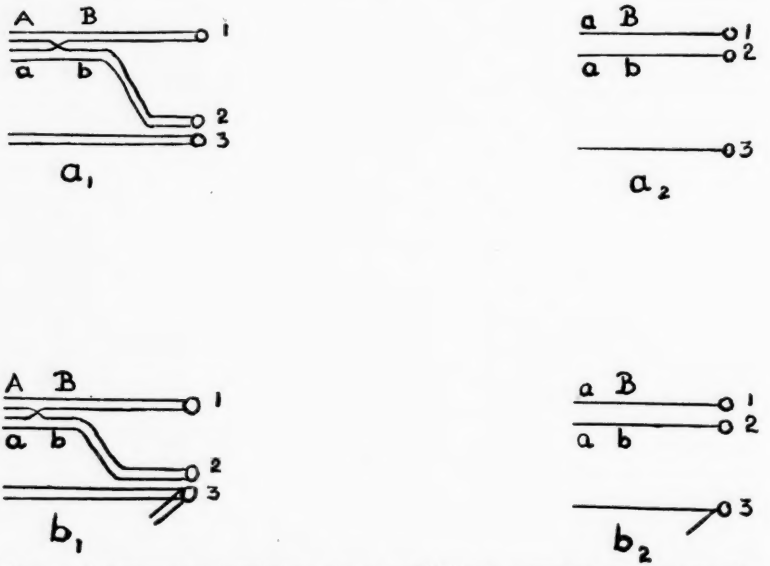

Fig. 2. A diagrammatic description of the possibility for crossing over between two Xs not pairing proximally in XXX females (a) and XXY females (b). 1 — pachytene. 2 — one of the disjunction types after second meiotic division.

COOPER's picture of the production of a X—$Y^S.Y^L$—X trivalent (COOPER, 1948).

It is perhaps worthwhile to note one consequence of abandoning BRIDGES' theory and adopting COOPER's. BRIDGES (1916) points out that »the resulting XY and X eggs (from heterosynapsis) must also be non-crossovers since there has been no XX synapsis which would give an opportunity for crossing over to occur. When these eggs are fertilized, they give rise only to regular offspring and should increase the non-crossover classes of the regular offspring», therefore a correction of the crossing over frequencies is necessary, *viz.* reducing the number of non-crossovers by twice the number of exceptions. STURTEVANT and BEADLE (1936), when they realised that the non-disjunction is not random, adopted another calculation method. They calculated the crossing over percentages from the total number of eggs produced, by adding twice the number of exceptional flies. When one adopts COOPER's hypothesis (1948) one realises that the exceptional eggs, both those giving rise to exceptional flies and those not developing, have no influence on the

crossover and non-crossover groups. The crossing over frequencies should, thus, be calculated directly from the numbers of flies counted without any correction (provided that really *all* non-disjunction happens in this manner, which appears to be at least very nearly so). This method of calculations is followed in the present work.

II. CROSSING OVER IN THE X CHROMOSOME WITH DIFFERENT AMOUNTS OF Y CHROMOSOME HETEROCHROMATIN

SCHULTZ and REDFIELD (1951) suggested that the increase of crossing over followed a change in the constitution of the normal diploid genotype is due to disturbances in the coiling, causing mechanical difficulties as well as by the action of heterochromatin. An increase in crossing over frequency is also observed in the XXY females. Now, this can accordingly be due to the mechanical disturbance of the unpaired Y chromosome or to the action of the heterochromatin of the Y chromosome. It was thought that it would be possible to distinguish between the two influences by adding to the normal female different amounts of the Y chromosome or of its fractions. It was also believed that through attaching Y chromosome fractions to the right arm of the X chromosome, the probable mechanical disturbance of pairing would be eliminated or reduced.

These experiments are far from being complete, and are intended to give a first insight into the problem. Further, as no standard gene marking was adopted, different experiments have sometimes different markers.

The first experiment was done in order to determine the normal distance between some genes used. Simultaneously, the crossing over values of the XXY and the XX sc^8.Y females have been determined. The results are given in Table 1. The left end of the X chromosome is not marked. The main part of the chromosome shows values lower than the

TABLE 1. *Crossing over values for XX, XXY and XXsc^8.Y females.*

	ec	ct	v	f	od	car	Total
Standard (BRIDGES and BREHME, 1944)	14,5	13,0	23,7	2,5	3,3		
XX females	10,6	10,7	16,7	2,1	4,7		2096
observed/standard	0,73	0,82	0,74	0,84	1,4		
XXY females	15,0	14,2	19,3	1,8	7,1		1756
	16,0	14,4	19,6	1,9	3,9		8788
XX sc^8. Y females	13,2	13,9	19,9	2,3	4,5		2638

TABLE 2. *The frequency of crossing over in the different experiments with XX.Y^S females. The numbers in brackets indicate the calculated expected frequencies for these intervals for XX females (see text). The limits of each interval are marked with* ×.

y, sc	w^c	ec	cv	ct	v	g	f	car	Total
×		(15,0)		× (10,7)	× (9,6)	×			
		19,5		14,2	9,6				1160
×	(10,0)		× (5,1)	× (10,7)	×	(16,7)	× (6,8)	×	
	8,9		5,0	13,6		25,3	3,4		1188
×	×	×	(11,0)	×	(28,0)		×		
0,0	2,2		14,5		36,9				360

standard, which can apparently not be completely due to the unobserved double crossovers between the markers, as the ratio 0,84 of observed to standard values is also found in the short part between f and od. The right end of the X chromosome shows a higher exchange frequency than the main interstitial part.

The substitution of the Y chromosome with sc^s.Y chromosome does not change significantly the exchange frequencies in the XXY females. Both cause increased exchange frequencies in the interstitial part of the X chromosome to about the same extent.

Three different experiments were done on exchange frequencies in the XX.Y^S females. The results are summarized in Table 2. It should be remembered that the XX females showed a reduced exchange frequency when compared with the standard values. Thus, expected normal values are calculated for the intervals studied here from the observed/standard ratio obtained in the experiment described above and in Table 1.

A further two series of experiments were done to compare exchange frequencies in the X.Y^S X.Y^L females and in the X.Y^S S.Y^L sc^s.Y females. The results are given in Table 3.

TABLE 3. *The frequencies of crossing over in two types of females, X.Y^S X.Y^L and X.Y^S X.Y^L sc^s.Y.*

	sc	ct	m	f	Totals
Calculated for XX females	(15,0)	(12,0)	(15,0)		
X.Y^S X.Y^L	20,8	13,7	16,3		307
	30,0	20,1	21,9		676
	11,3	17,3	23,3		382
	17,6	11,9			210
X.Y^S X.Y^L sc^s.Y	21,8	16,7	17,1		257
	29,8	15,9	28,8		319

TABLE 4. *Crossing over frequency in the interval cut to forked in the different chromosomal combinations studied.*

	XX	$X.Y^{S}X$	$X.Y^{S}$ $X.Y^{L}$	XXY	$XXsc^{8}.Y$	$X.Y^{S}$ $X.Y^{L}$ sc^{8} Y
	27,4	38,9	30,0	33,5	33,8	33,8
		36,9	42,0	34,0		34,6
			40,6			
average	27,4	37,6	37,5	33,7	33,8	34,2

It can be seen that in all females containing parts of, or the whole Y chromosome, an increased exchange frequency was found. Further, the increase in exchange frequency seems to be of the same magnitude for all experiments, irrespective of the »amount» of Y chromosome added and of the Y being attached or free. It may be that the presence of a free Y chromosome somewhat decreases the increase effect obtained by Y chromosome parts attached to the right arm of the X chromosome. These points are more obviously seen in Table 4, where the results are summarized for the ct-f interval which was marked in all the experiments.

Assuming that these results will be verified by further experiments, that it is not the mere presence of a chromosome body which works here to cause an increase in exchange frequencies, the factor which increases the exchange frequencies seems to be the presence of Y chromosome heterochromatin. The lack of proportionality between the amount of heterochromatin and exchange increase, indicates that the effect of the presence of Y chromosome heterochromatin is an all- or non-effect, or that there is a special part in the heterochromatin (presumably in the Y^{S}) which is responsible for the observed effect. That Y chromosome increases the exchange frequency was also observed by SANDLER (1954), but he found this effect to be confined to the acrocentric compound X chromosome. Further he suggested »that Y chromosome heterochromatin has a different homology with X chromosome

TABLE 5. *The frequency of secondary non-disjunction in different chromosomal combinations studied.*

Chromosomal combinations	Number exceptional flies	Total flies	% secondary non-disjunction	P homogeneity with XXY	P homogeneity with $XXsc^{8}.Y$
XXY	526	12805	4,11	—	—
XX $sc^{8}.Y$	477	7529	6,34	$> 0,001$	—
$XX.Y^{S}$ $sc^{8}.Y$	49	1362	3,60	0,4	$> 0,001$
$X.Y^{S}$ $X.Y^{S}$ $sc^{8}.Y$	24	791	3,03	0,2—0,1	$> 0,001$
$X.Y^{S}$ $X.Y^{L}$ $sc^{8}.Y$	5	704	0,71	$> 0,001$	$> 0,001$

heterochromatin than X chromosome heterochromatin has with itself». This appears also here to be the case, as the addition of any amount of Y heterochromatin produces the effect. SANDLER (1954) observed that the attachment of the Y^L to the right arm of the X chromosome was necessary for the heterochromatin exchange needed for the production of the reversed acrocentric. If this is also a typical function of the Y chromosome heterochromatin, this special exchange will also be produced in the XXY females, but there the products will usually remain unnoticed, as they are not distinguishable from the other matroclinous daughters of such females.

Table 5 gives the frequencies of secondary non-disjunction for a number of combinations studied. It can be seen that the substitution of the Y chromosome by a sc^s.Y chromosome, while not causing an increase in the exchange frequency (Table 1), causes a significantly greater amount of secondary non-disjunction. According to COOPER's theory (1948), this means that the »affinity» of the sc^s.Y towards the X chromosome has increased. Not less striking is the observation that when the Xs have part of the Y chromosome attached to them the secondary non-disjunction frequency decreases significantly in proportion to the size of the Y chromosome part attached to the right arm of the X chromosome. It seems as if the »elongated» X chromosome has now a greater part of it ready to pair with the other X-chromosome, thus competing more successfully with the sc^s.Y chromosome.

III. DETACHMENT OF THE ATTACHED X CHROMOSOME

As mentioned above, detachments of attached X chromosomes were searched for in order to get the chromosome necessary for this series of experiments. Two such crosses were performed: Cross *a* was made in order to obtain X.Y^S chromosomes by crossing y.ymf.=/sc^s.Y females to yB/sc^s.Y males (the sc^s is attached to the long arm of the Y chromosome). In cross *b* y.ymf.=/sc^{S1}.Y^L females were mated to y ac sc ct f.Y^S/sc^{S1}.Y^L males in order to obtain the X.Y^L chromosome.

14134 flies were counted in cross *a*. 9 detachments being obtained, *viz.* 0,064 %, but these 9 flies appeared on 5 different occasions. As the cross was made in mass cultures, there is no definite proof of clusters being from single females when on one occasion 2 detachments and on the other 4 were found. It is nevertheless suggestive of clustering, and fits COOPER's claim (1946) that the detachments are due to a mitotic event and not a meiotic. If we base the calculation on only 5 such

events, the detachment frequency is 0,035 %, which is in good agreement with COOPER's results. This is even more emphatically seen in cross *b* where on one occasion 23 females and 13 males were detachments of the same type. If here every case is counted as a different detachment, one gets 39 detachments in 8605 flies, *viz.* 0,45 % (this is a minimum value, as one type of detachments can not be recognized in the females). Taking each cluster as a different case one gets 4 detachments or 0,047 %. Such a great cluster shows that the detachment occurred far prior to the oocyte stage in which PARKER (1953) found his X-ray induced detachments.

The frequency of primary non-disjunction was nearly the same in the two crosses, 0,18 % in cross *a* and 0,13 % in cross *b*.

For help in the preparation of the text I wish to express my sincere thanks to Professor G. BONNIER and Miss E. MACKENDRICK.

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OXYGEN AND THE PRODUCTION OF CHROMOSOME ABERRATIONS BY CHEMICALS AND X-RAYS

By *B. A. KIHLMAN*

INSTITUTE OF PHYSIOLOGICAL BOTANY, UNIVERSITY OF UPPSALA, AND THE WALLENBERG LABORATORY FOR EXPERIMENTAL CYTOLOGY, INSTITUTE FOR CELL RESEARCH, KAROLINSKA INSTITUTET, STOCKHOLM, SWEDEN

I. INTRODUCTION

IT is now a well-established fact that the presence of oxygen during exposure to X-rays drastically promotes the production of mutations and chromosome breakage. As an explanation of the oxygen effect in the case of X-rays, the radical theory has now been generally accepted. According to this theory, the effect of X-rays in aqueous systems is mediated by active radicals resulting from the radiodecomposition of water. When dissolved oxygen is present, the HO_2 radical is formed, and this radiation product is believed to be the important one in the induction of mutation and chromosome breakage. The facts on which this radiochemical interpretation is founded have recently been summarized and discussed by GRAY (1953) and by GILES (1954).

With this explanation of the oxygen effect in the case of X-rays in mind, an effect of oxygen on the production of chromosome aberrations by chemicals would not be expected, since these agents probably act directly on organic molecules and not through the intermediary of water (GRAY, 1953). The effects of chemicals of the mustard type seem, in fact, to be unaffected by oxygen tension during treatment. No direct evidence for the absence of an oxygen effect in the production of chromosome aberrations by mustards has yet been published. However, the production of sex-linked lethals in *Drosophila* by mustard gas is unaffected by the oxygen tension during treatment (AUERBACH and MOSER, 1951), as is the growth inhibition of *Vicia* roots produced by nitrogen mustard (READ, 1950). Both these effects are probably closely related to chromosome structural damage. On the other hand, when the effect of oxygen on the production of chromosome aberrations by the purine derivative 8-ethoxycaffeine was studied, it was found, surprisingly

enough, that oxygen drastically influenced the radiomimetic effect (KIHLMAN, 1954).

In the present paper, results are reported which have emerged from a comparative study of the influence of oxygen on the frequency of chromosome aberrations produced by 8-ethoxycaffeine, X-rays, and by nitrogen mustard.

II. MATERIAL AND METHODS

Experimental material consisted of root tips of a Swedish variety (»Primus») of the broad bean, *Vicia faba*. Treatments were applied to lateral roots. The seedlings were grown in the dark.

The treatments with 8-ethoxycaffeine (EOC) were, as a rule, performed with a 10^{-2} M solution of the compound. Regarding length of time of treatment, temperature, and duration of recovery, two kinds of experiments were performed. In one series of experiments, roots were treated for 1 $^3/_4$ hr. with EOC at 20° C, and were then transferred to water and fixed 5 $^1/_2$ hr. subsequent to the EOC treatment. In the other type of experiments, roots were treated for periods varying between $^1/_2$ and 8 hr. at 10° C, the recovery period after treatment being 19 hr. The low treatment temperature was used because at 10° C the toxic effect of EOC, which at 20° C is considerable, is almost eliminated, while, on the other hand, the production of chromosome aberrations is promoted.

Nitrogen mustard, di-(2-chloroethyl) methylamine hydrochloride, was used in a concentration of 10^{-5} M, the period of treatment being $^1/_2$ hr. and the treatment temperature 10° C. Roots were fixed after a 24-hour recovery in water.

Two series of X-ray experiments were performed. In the first series, the beans were irradiated on glass tubes with their roots immersed in water. The irradiations were given at 145 kV, filter 1 mm Al, intensity 27 r/min. These irradiations (108 and 216 r) were carried out at the Institute of Radiophysics, Stockholm. The second series of X-ray treatment were given at the Institute of Plant Systematics and Genetics, Royal Agricultural College, Uppsala.[1] In these experiments, the roots were irradiated in closed glass vessels. The roots were given 250 r at 170 kV and at an intensity of 50 r/min. In both series or experiments, the roots were irradiated at room temperature, *i. e.* c. 20° C.

[1] I am very much indebted to Dr. ARNE FORSSBERG, and to Dr. BERTIL RASMUSON, who performed the irradiations in Stockholm and Uppsala, respectively.

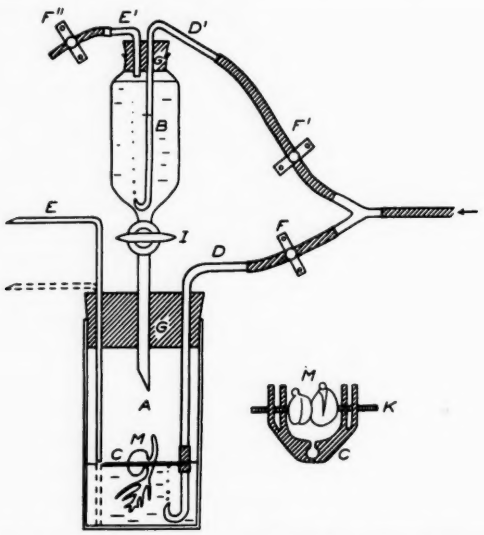

Fig. 1. Apparatus for treatments of bean roots with chemicals at varying oxygen concentrations.

The temperature during the recovery period was 20° C after treatments with chemicals as well as with X-rays. All experiments were performed in the dark. As a rule, the roots were treated with 0,05 per cent colchicine for $3^1/_2$ hr. before fixation.

As long as the experiments were carried out at only three different oxygen concentrations, namely 0 per cent (nitrogen gas), 21 per cent (air), and 100 per cent, they were performed in the same way as in connection with the *Allium* experiments described previously. Later, when the effect of intermediate oxygen concentrations was tested, it proved to be necessary to construct special treatment vessels to be sure that the oxygen concentration in the solution did not vary during treatment as a result of back-diffusion of oxygen from the air. The treatment vessel is shown in Fig. 1. It consists of a cylindrical glass jar (A) 10 cm high and 5 cm in diameter, the glass being 2 mm in thickness. The jar is closed by a rubber stopper (G) carrying an entry tube (D), a movable exit tube (E), and a separatory funnel (B). The funnel, which is also closed with a rubber stopper (G′) through which pass entry and exit tubes (D′ and E′), is filled with the treatment solution. Some days before the treatment, a thin strip (K) of plastic is passed through the

cotyledons of the beans (M) to be treated. Fig. 1 shows how the beans are attached to the entry tube D by means of this strip and a plastic plate (C).

The jar is filled with water and the exit tube E is pushed down to the bottom of the jar. The next step is to replace slowly the water in the jar by the gas mixture in question, at the same time keeping it bubbling through the treatment solution in the funnel. The gas flow into A and B through the tubes D and D′ is regulated by means of the screen clamps F and F′. When A is completely filled with gas, the tube E is moved upwards to the position it has in Fig. 1. The exit tube E′ is closed by means of the screen clamp F″ and by turning the stopcock I, the treatment solution in B is allowed to flow into A until the surface of the solution is on a level with C. During the whole treatment, gas is bubbled through the treatment solution in A.

Roots from two beans were always treated simultaneously. The root tips were fixed in alcohol-acetic acid, 3:1, prepared as Feulgen squashes, and made permanent according to the dry-ice method. Two slides were made for each bean and two or three tips were used for each slide. At least 100 cells were analysed for each treatment. The aberrations were scored according to the terminology of THODAY (1951). In general, values for single chromatid breaks are not included in the tables, since it is difficult to obtain reliable scores of this type of change.

III. RESULTS

1. The production of structural chromosome changes in Vicia by 8-ethoxycaffeine (EOC)

Before the influence of oxygen on the production of chromosome aberrations by X-rays, nitrogen mustard, and EOC is described, a short account of the effect of EOC on the root tip chromosomes of *Vicia faba* will be given. Previously, the effect of EOC in *Vicia* has been briefly described by KIHLMAN and LEVAN (1951), and by MCLEISH (1954).

The interphase chromosomes in *Vicia*, like those in *Allium* (KIHLMAN, 1955 a), are most sensitive to breakage by EOC at the end of the resting stage. At this stage, aberrations involving only one of the two chromatids of a chromosome predominates, whereas aberrations of the isolocus type (involving both chromatids) are most common among the chromosome changes induced at the early interphase. So far as interphase sensitivity is concerned, the effect of EOC is more similar to that of X-rays than it is to the radiomimetic effect of nitrogen mustard (REVELL, 1953).

TABLE 1. *Chromosome aberrations obtained in Allium and Vicia 5 1/2 hours after treatment with 8-ethoxycaffeine (EOC) and X-rays.*

Material	Treatment	Recovery period, hours	Number of metaphases analysed	Abnormal metaphases per cent	Aberrations per 100 cells			Chromatid exchanges / Chromatid breaks
					Chromatid breaks	Isolocus breaks	Cromatid exchanges	
Allium cepa	EOC 10^{-2}M, 50 min.	5 1/2	115	54,8	54	23	5	0,1
	X-rays, 75 r. (20,1 r/min.)	5 1/2	200	83,5	103,5	23,5	28	0,3
Vicia faba	EOC 10^{-2}M, 1 3/4 hr.	5 1/2	400	64	14,5	19	85	5,9
	X-rays 108 r. (27 r/min.)	5 1/2	200	86,5	82,5	31	51	0,6

The ratios between *chromatid exchanges* and *chromatid breaks* obtained 5 1/2 hr. after treatment are, however, quite different from those obtained after X-irradiation, and also different from those produced by the same chemical in *Allium cepa*. As appears from Table 1, EOC produces in the late interphase cells of *Vicia* very few chromatid breaks and a large number of exchanges, the exchange/break ratio being 5,9. The corresponding ratio obtained after X-irradiation is 0,6. In *Allium* the effect of EOC is the opposite, a large number of chromatid breaks and only a few exchanges being produced. The exchange/break ratio obtained after exposure to X-rays is of about the same order of magnitude in *Allium* as in *Vicia*.

In the early interphase cells of *Vicia* root tips, EOC produces relatively less *exchanges* and more *isolocus breaks* than do X-rays (Fig. 3). A large proportion, usually between 50 and 80 per cent, of the EOC-induced isolocus breaks are localized in the nucleolar constriction of the two long chromosomes (Table 2; Plate I, Fig. 4). Similarly, the points of exchange are also frequently situated in the nucleolar constrictions when chromatids in the two long chromosomes of *Vicia* are interchanged as a result of treatments of early interphase cells with EOC (Table 2; Plate I, Fig. 1). About 30 per cent of the exchanges obtained after a recovery period of 19 hours are of this type. Most of the

Plate I. Figs. 1—4. — Metaphases (all colchicine treated) from root tip squashes of *Vicia faba*, showing the major types of aberration induced by 8-ethoxycaffeine. — Fig. 1. Chromatid interchange between the two long chromosomes. The points of exchange are in the nucleolar constrictions. — Fig. 2. Chromatid interchange between long and short chromosomes. — Fig. 3. *a.* Chromatid interchange between long and short chromosomes. One of the points of exchange is in the nucleolar constriction of the long chromosme; *b.* Isolocus break in a short chromosome. — Fig. 4. *a.* Isolocus/chromatid exchange (incomplete) between short chromosomes; *b.* Isolocus break in a short chromosome; *c.* Isolocus breaks in the nucleolar constrictions of the two long chromosomes. — Alcohol-acetic acid, 3 : 1. — Feulgen squashes. — ×1700.

TABLE 2. *The influence of dose, temperature and duration of recovery on the frequency of chromosome aberrations produced by 8-ethoxycaffeine.*

Concentration of EOC M	Period of treatment, hours	Treatment temperature °C	Recovery period, hours	Number of metaphases analysed	Abnormal metaphases per cent	Abberations per 100 cells							
						Chromatid breaks	Isolocus breaks		Cromatid exchanges		Isolocus/chromatid exchanges	Isolocus/isolocus exchanges	Minutes
							in nucleolar constriction	in other places	in nucleolar constriction	in other places			
10^{-2}	1 3/4	28	5 1/2	100	21	14	3	2	0	6	0	0	—
5×10^{-3}	1 3/4	20	3 1/2	100	9	2	1	4	0	2	0	0	—
5×10^{-3}	1 3/4	20	5 1/2	300	9	1	1,3	2,3	1,3	3,7	0	0	—
10^{-2}	1 3/4	20	5 1/2	100	69	11	5	8	0	90	3	0	—
10^{-2}	1 3/4	20	9	100	29	4	5	7	0	26	1	0	—
10^{-2}	1 3/4	20	17	100	11	0	12	0	0	0	0	0	—
5×10^{-3}	2	10	19	200	21	0	21	0,5	1	0	0	0	0
5×10^{-3}	4	10	19	100	40	0	33	13	1	0	2	0	0
10^{-2}	1/2	10	19	200	2	0	1	1	0	0	0	0	0
10^{-2}	1	10	19	200	14,5	0,5	7,5	7	0,5	1,5	0,5	0	0,5
10^{-2}	2	10	19	200	35,5	0,5	29,5	8,5	1,5	2	0	0	0,5
10^{-2}	4	10	19	300	61	1,7	49	25	1,5	15	3,5	0	0
10^{-2}	8	10	19	200	88	3	80,5	44	8,5	16,5	5,5	1,5	7

remaining 70 per cent are interchanges of the ordinary type shown in Plate I, Fig. 2; but some interchanges have been obtained where one point of exchange is situated in the nucleolar constriction and the other is not (Plate I, Fig. 3). *Isolocus/chromatid exchanges* (Plate I, Fig. 4) occur after EOC treatments although with a low frequency, whereas *isolocus/isolocus exchanges* have been obtained only exceptionally. *Minutes* occur with a low frequency compared with that obtained after treatments with nitrogen mustard and X-rays (Tables 2, 3, and 4).

When the treatments are performed at 10° C and the roots fixed 19 hours afterwards, the yield of aberrations obtained is roughly doubled when the period of treatment or the concentration of EOC is doubled, *i. e.* the effect is proportional to the dose (Table 2; Fig. 2). However, a lower yield than expected is obtained after periods of treatment of one hour or less.

When the roots are fixed $5^1/_2$ hr. after $1^3/_4$ hr.-treatments at 20° C, the yield of aberrations obtained shows larger deviations from the dose-proportionality rule. Thus, 5×10^{-3} M EOC produces practically no effect, although after treatments with 10^{-2} M a very high frequency of aberrations is obtained.

The temperature during treatment was found to influence the radiomimetic effect of EOC in *Vicia* in a similar way as it influences that in *Allium* and *Pisum* (KIHLMAN, 1951, 1952), *i. e.* the effect is increased when the temperature is lowered. The toxic effect of EOC, on the other hand, decreases when the temperature is lowered.

2. Oxygen and chromosome breakage

X-rays. — The effect of X-rays on the root tip chromosomes of *Vicia faba* has been investigated by several workers, *e. g.*, DARLINGTON and LA COUR (1945), THODAY (1951) and REVELL (1953).

The discovery of THODAY and READ (1947) that a given dose of X-rays produces about three times more chromosome aberrations in an oxygen than in a nitrogen atmosphere was also made with *Vicia* as experimental material. The experiments of these authors were performed only at two oxygen levels, however. For comparison with the oxygen effect in the case of EOC, it was desirable to have the relation between oxygen concentration and yield of chromosome aberrations explored in greater detail. Consequently, an experiment was carried out in which exposures to a single X-ray dose — 250 r at 50 r/min. — were made at six different percentages of oxygen in the gas phase in the treatment vessel, namely

0,3 (nitrogen), 2,7, 5,7, 10,6, 21 (air), and 99,7 (oxygen) per cent. In Fig. 3 the yields of exchanges and isolocus breaks are plotted against the concentration of dissolved oxygen. This concentration was calculated according to HENRY's law, the value of K being obtained from the Handbook of Chemistry and Physics, 30th Ed. The yield of aberrations per cell was at each oxygen concentration calculated on the basis of 200 analysed metaphases.

The results of these experiments resemble those obtained by GILES and BEATTY (1950) in *Tradescantia* in that there is a rapid increase in the frequency of aberrations between 0 and 10 per cent oxygen. Some differences may be noted, however. In *Tradescantia*, the increase is more gradual after 10 per cent oxygen and shows a definite leveling-off at around 21 per cent. Between 21 and 100 per cent, there is a slight but still quite significant increase in aberration frequency. In the present experiments, no further increase was obtained in aberration frequency between 21 and 100 per cent oxygen, as appears from Fig. 3. The maximum effect of oxygen in increasing the aberration frequency was reached somewhere between 10 and 21 per cent. The difference between the effect in nitrogen and oxygen seems also to be somewhat larger than that reported by THODAY and READ and by GILES and BEATTY.

In another series of X-ray experiments, the effect of the cytochrome oxidase inhibitor, sodium azide (NaN_3), was tested. Previous attempts to relate the influence of anaerobiosis on radiosensitivity to the oxidized or reduced state of enzymes concerned with respiration have given very contradictory results. BACQ and HERVE (1951) obtained protection of mice against a lethal dose of X-rays by cyanide, azide and malononitrile; and a protection by cytochrome oxidase inhibitors against X-ray damage was also observed by LASER (1954) working with bacteria. SOBELS (1955), on the other hand, found that pretreatment with cyanide and azide significantly increased the frequency of sex-linked lethals in *Drosophila*. KING, SCHNEIDERMAN and SAX (1952) obtained an increased frequency of chromosome aberrations in *Tradescantia* microspores when the irradiations were performed in the presence of carbon monoxide, a specific inhibitor of cytochrome oxidase, whereas MIKAELSEN (1954) found that sodium cyanide decreased the yield of aberrations produced by γ-irradiation in *Tradescantia* root tips.

The results of the present experiments appear in Table 3. In the root tips irradiated with 216 r, a somewhat lower yield of aberrations was obtained when the irradiation was performed in the presence of sodium azide. In the roots irradiated with 108 r, on the other hand, no signif-

TABLE 3. *The yields of isolocus breaks, exchanges, and minutes obtained 20 hr. after X-ray irradiation in nitrogen, in air, and in the presence of sodium azide. (Intensity 27 r./min; temperature during treatment and recovery c. 20° C.)*

Pre-treatment at 20°C	X-ray dose in r	Treatment atmosphere	Concentration of NaN_3 M	Number of metaphases analysed	Abnormal metaphases per cent	Aberrations per 100 cells		
						Isolocus breaks	Exchanges	Minutes
—	108	nitrogen	—	100	10	5	5	2
—	108	air	—	200	50	28,5	25	13,5
10^{-3}M NaN_3, 30 min.	108	air	10^{-3}	200	54,5	36,5	25	14,5
—	216	nitrogen	—	100	28	16	12	6
—	216	air	—	100	73	63	61	27
4×10^{-4}M NaN_3 30 min.	216	air	4×10^{-4}	100	59	48	41	19

icant difference is obtained between irradiations performed in the presence or absence of the respiration inhibitor.

It is therefore concluded that the frequency of chromosome aberrations produced in *Vicia* by X-irradiation probably is unaffected by sodium azide. In any case, there cannot be any protection comparable to that of anaerobiosis. Since, in roots, the respiration is almost completely inhibited by low concentrations of sodium azide (MARSH and GODDARD, 1939), the chromosome damage produced by X-rays seems to be independent of respiration.

Nitrogen mustard. — In investigations of the radiomimetic effect of nitrogen mustard, root tips of *Vicia faba* have previously been used as experimental material by FORD (1949) and REVELL (1953).

In the present experiments, the influence of oxygen on the radiomimetic effect of nitrogen mustard was tested at five different concentrations of oxygen in the gas phase, namely 0,3, 5,7, 10,6, 21, and 99,7 per cent. As appears from Table 4 and Fig. 3, the oxygen concentration during treatment does not seem to affect significantly the yield of aberrations. The results are thus in agreement with those of AUERBACH and MOSER (1951) and READ (1950).

The possibility that the radiomimetic effect of nitrogen mustard is dependent on oxygen tension cannot yet be completely ruled out, however. Facts indicate that, in the case of nitrogen mustard, there is a time interval between treatment and the production of chromosome aberrations (REVELL, 1953). If oxygen only influences »the events in the

TABLE 4. *The frequencies of isolocus breaks, exchanges and minutes obtained 24 hours after treatments with nitrogen mustard (10^{-5} M, $^1/_2$ hr., 10° C).*

Concentration of oxygen in surrounding gas, per cent	Approximate concentration of dissolved oxygen (at 10°C), μM	Number of metaphases counted	Abnormal metaphases per cent	Aberrations per 100 cells		
				Isolocus breaks	Exchanges	Minutes
0,3	5	100	86	127	32	48
5,7	95	100	85	110	31	56
10,6	180	100	83	96	24	44
C. 21	360	100	92	122	27	47
99,7	1700	100	82	86	33	47

chromosomes which produce the aberrations themselves» (REVELL, 1953) an oxygen effect in the case of nitrogen mustard should be obtained not during, but some time after, the treatment. This possibility has also been mentioned by READ (1950).

A preliminary experiment was performed to test if the aberration frequency produced by a given treatment of nitrogen mustard is influenced by anaerobic periods during recovery. The result of the experiment was negative.

8-ethoxycaffeine (EOC). — Preliminary experiments with *Allium* as experimental material indicated that the production of chromosome aberrations by both EOC and X-rays is influenced by oxygen tension in a similar way. The oxygen dependence could therefore be added to the many similarities existing between the effects of EOC and X-rays (KIHLMAN, 1955 a). The more detailed experiments, which were carried out with *Vicia* as test material, have, however, revealed important differences in the way in which the oxygen effect is operating in the two cases.

It appears from Table 6 and Fig. 2 that EOC in the absence of oxygen produces practically no effect. In experiments where it was certain that the treatment solution was free from dissolved oxygen, treatments with 10^{-2} M EOC for periods up to eight hours did not produce any effect. It seems, therefore, as if the presence of oxygen not only promotes the production of aberrations, as in the case of X-rays, but is a prerequisite for inducing chromosome damage by EOC.

As in the case of X-rays and nitrogen mustard, the effect of EOC has also been tested at different oxygen concentrations. It appears from Fig. 3 that the shape of the curves thus obtained for exchanges and

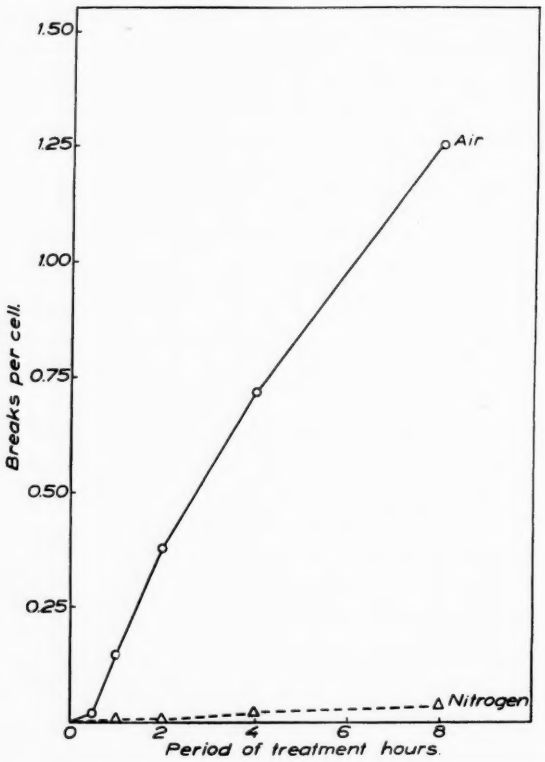

Fig. 2. Graph showing the yield of isolocus breaks obtained 19 hours after treatments with 10^{-2} M 8-ethoxycaffeine in air and in nitrogen.

isolocus breaks is very similar to that obtained after irradiation with 250 r of X-rays, with the exception that EOC, produces no effect in the absence of oxygen. (All points are based on 200 analysed metaphases.) The fact that the shapes of the EOC and X-ray curves are so similar is somewhat surprising, considering that the oxygen effects in the two cases probably have entirely different explanations.

In preliminary experiments, the frequency of aberrations produced by EOC was found to be unaffected by pre- and post-treatment exposures of the material to anaerobic conditions (KIHLMAN, 1955 b). In these experiments, the roots were fixed $5^1/_2$ hr. after the EOC treatment. Later,

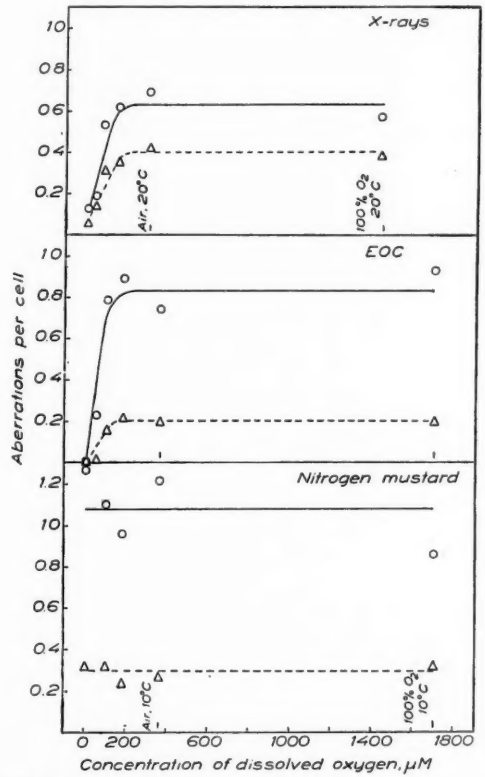

Fig. 3. The yields of isolocus breaks (o———o) and exchanges (△ - - - - △) obtained one day after treatments with EOC (10^{-2} M, 4 hr., 10° C), nitrogen mustard (10^{-3} M, $^1/_2$ hr., 10° C), and X-rays (250 r at 50 r/min., 20° C) at different oxygen concentrations. EOC=8-ethoxycaffeine.

the experiments were repeated, but with the difference that the roots were treated at 10° C and fixed 19 hr. after the treatment. In these experiments, the effect of EOC was significantly decreased by pre-treatment exposures to pure nitrogen (Table 5). Post-treatment exposures had no effect on the total aberration frequency obtained, but mitotic counts revealed that the recovery from the mitotic depression produced by EOC was delayed. These experiments were repeated with identical results.

It may be noted in this connection that the frequency of chromosome

TABLE 5. *The yields of isolocus breaks and exchanges obtained 19 hours after treatments with 10^{-2} M 8-ethoxycaffeine (EOC), when the material was exposed to aerobic or anaerobic conditions during the treatments or immediately before or after the treatments.*

Expt. No.	Pre-treatment (2 hr. 20°C) in	EOC-treatment (2 hr. 10°C) in	Post-treatment (2 hr. 20°C) in	Number of metaphases analysed	Abnormal metaphases per cent	Aberrations per 100 cells		
						Isolocus breaks		Exchanges
						in nucleolar constriction	in other places	
61	air	air	air	100	36	26	11	5
63	»	»	»	100	39	32	10	3
				mean	37,5	29	10,5	4
45	»	nitrogen	»	200	1	0,5	0	0,5
61	nitrogen	air	»	100	8	5	6	0
63	»	»	»	100	13	9	1	3
				mean	10,5	7	3,5	1,5
61	air	»	nitrogen	100	34	20	15	7
63	»	»	»	100	28	15	17	9
				mean	31	17,5	16	8

TABLE 6. *The effect of oxygen tension and of various chemicals on the frequencies of isolocus breaks and exchanges obtained after treatments with 10^{-2} M 8-ethoxycaffeine. DNP=2.4-dinitrophenol; ONP=orthonitrophenol.*

Pre-treatment	Duration of treatment hours	Treatment-atmosphere	Treatment temperature °C	Recovery period hours	Chemical added		Number of metaphases analysed	Aberrations per 100 cells		
					formula, abbreviation	concentration M		Isolocus breaks		Exchanges
								in nucleolar constriction	in other places	
—	1 3/4	air	20	5 1/2	—	—	200	4,5	10	94,5
—	1 3/4	nitrogen	20	5 1/2	—	—	400	0,5	1,3	2,75
—	1 3/4	»	20	5 1/2	H_2O_2	10^{-3}	200	0	0,5	0,5
—	1 3/4	»	20	5 1/2	»	2×10^{-3}	200	0,5	2,5	26,5
—	1 3/4	»	20	5 1/2	»	5×10^{-3}	200	1	12,5	70
—	1 3/4	air	20	5 1/2	KCN	10^{-3}	100	0	0	0
—	1 3/4	»	20	5 1/2	NaN_3	2×10^{-4}	200	1	16,5	40
—	1 3/4	»	20	5 1/2	»	4×10^{-4}	100	0	0	1
—	1 3/4	»	20	5 1/2	»	8×10^{-4}	100	0	2	0
—	1 3/4	»	10	5 1/2	—	—	150	4,7	22,2	59,3
2×10^{-4}M DNP, 5 min., 18°C	1 3/4	»	10	5 1/2	DNP	10^{-4}	200	1	4,5	15
—	2	»	10	19	—	—	200	29,5	8,5	3,5
—	2	nitrogen	10	19	—	—	200	0,5	0	0,5
2×10^{-4}M DNP, 20 min., 20°C	2	air	10	19	DNP	10^{-4}	150	0	4	0
—	4	»	10	19	—	—	200	49	25	20
—	4	nitrogen	10	19	—	—	300	1,3	1,3	0,8
10^{-3}M KCN, 30 min., 10°C	4	air	10	19	KCN	$7,5\times10^{-4}$	100	2	0	0
4×10^{-4}M NaN_3, 10 min., 10°C	4	»	10	19	NaN_3	4×10^{-4}	100	1	0	0
4×10^{-4}M NaN_3, 30 min., 10°C	4	»	10	19	»	4×10^{-4}	200	5	2	2
2×10^{-4}M DNP, 15 min., 18°C	4	»	10	19	DNP	10^{-4}	100	15	2	0
2×10^{-4}M DNP, 30 min., 20°C	4	»	10	19	»	10^{-4}	200	3	2	0
2×10^{-4}M ONP, 15 min., 18°C	4	»	10	19	ONP	10^{-4}	100	49	18	14
2×10^{-4}M ONP, 30 min., 20°C	4	»	10	19	»	10^{-4}	100	53	13	10

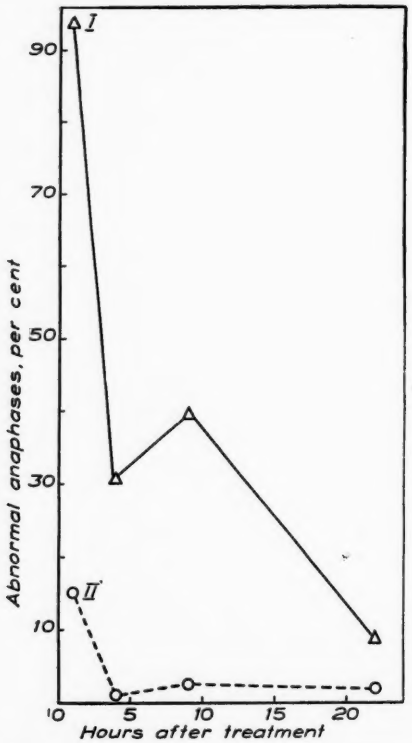

Fig. 4. The effect of sodium azide on the percentage of abnormal anaphases obtained after treatments with 8-ethoxycaffeine (EOC). I=10^{-2} M EOC, 1 3/4 hr., 20° C; II=10^{-2} M EOC, 1 3/4 hr., 20° C, 4×10^{-4} M NaN_3.

aberrations produced by X-rays in *Tradescantia* is unaffected by pretreatment exposures to anaerobic conditions (GILES and RILEY, 1950).

Hydrogen peroxide (H_2O_2) was tested in connection with the EOC experiments, because it has been suggested that this chemical is implicated in the initiation of chromosome structural damage by X-rays in the presence of oxygen (THODAY and READ, 1949).

H_2O_2 did not produce any chromosome aberrations when tested alone, but it was found that it can replace oxygen as a factor necessary for the radiomimetic effect of EOC. When roots were treated with 10^{-2} M EOC in the absence of oxygen, but with 5×10^{-3} M H_2O_2 in the treat-

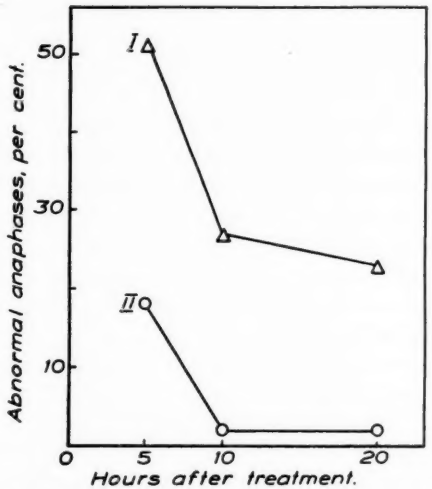

Fig. 5. The effect of 2.4-dinitrophenol (DNP) on the percentage of abnormal anaphases obtained after treatments with 8-ethoxycaffeine (EOC). I=10^{-2} M EOC, 2 hr., 10° C; II=10^{-2} M EOC, 2 hr., 10° C, 10^{-4} M DNP.

ment solution, an aberration frequency of the same magnitude as that produced under aerobic conditions was obtained (Table 6).

The effect of cytochrome oxidase inhibitors was also tested in the EOC experiments. In addition to sodium azide, the inhibitor used in the X-ray experiments, the effect of potassium cyanide was tested. However, since the latter compound was found to increase the mitotic inhibition produced by EOC, most of the experiments were performed with sodium azide.

As appears from Table 6 and Fig. 4, the radiomimetic effect of EOC is suppressed by sodium azide and potassium cyanide as much as by anaerobic conditions. Thus, in this respect the effect of EOC differs from that of X-irradiation, which was found to be unaffected by sodium azide. It is concluded that the oxygen effect in the case of EOC operates by way of the respiratory chain, and that molecular oxygen has no effect if the cytochrome system does not function.

In a number of aerobic experiments, 2.4-dinitrophenol (DNP), which is known to uncouple phosphorylation from oxidation (LOOMIS and LIPMANN, 1948; CROSS *et al.*, 1949), was added to the treatment solutions in a concentration of 10^{-4} M. As appears from Table 6 and Fig. 5, the

frequency of chromosome aberrations produced by EOC is reduced by DNP almost as much as by the absence of oxygen. However, an effect as drastic as that produced by anaerobic conditions was obtained with DNP only if the treatments were performed at 10° C and if the roots were exposed to DNP not only during the EOC treatment, but also before the treatment for about half an hour. When *ortho*-nitrophenol (ONP) was substituted in the treatment solution for DNP, no reduction in the frequency of aberrations was obtained. ONP does not uncouple phosphorylation (CROSS *et al.*, 1949).

The experiments with DNP indicate that components containing energy-rich phosphate bonds are involved in the production of chromosome aberrrations by EOC.

Finally, it should be mentioned that the stickiness effect produced in dividing cells by EOC is similarly affected by oxygen tension and chemicals as the structural chromosome changes. In the absence of oxygen as well as in the presence of cytochrome oxidase inhibitors or of DNP, practically no stickiness is produced by an EOC-treatment which otherwise should have resulted in an extreme stickiness.

IV. DISCUSSION

Different mechanisms are probably involved in the production of chromosome aberrations by X-rays, EOC, and nitrogen mustard. This is suggested by the following facts: (1) that the effects of EOC and X-rays are dependent on oxygen tension during treatment, whereas the radiomimetic effect of nitrogen mustard seems to be unaffected by oxygen, and (2) that the effect of EOC, in contrast to that of X-rays, is dependent on respiration.

As regards EOC, the results obtained can also give some information as to the mechanism itself.

We know now that, in this case, the oxygen effect operates by way of the respiratory chain and that molecular oxygen has no effect if the cytochrome system does not function. The influence of H_2O_2 in promoting the production of chromosome aberrations is probably due to the fact that molecular oxygen is produced *in vivo* from H_2O_2 as a result of catalase action, or that an oxidation of cytochromes by H_2O_2 is catalyzed by peroxidases.

The fact that the radiomimetic effect of EOC is influenced by pretreatment exposures to anaerobic conditions rules out the possibility that EOC is inactive as such, but that in the presence of oxygen it is

oxidized to an active compound by the way of the respiratory chain. For the same reason, the possibility can be eliminated that some compound with radiomimetic properties is formed when cells respire in the presence of EOC. However, the effect of pre-treatment exposures to anaerobic conditions becomes understandable if some compound normally formed during respiration is involved in the production of chromosome aberrations by EOC. Respiration is known to be coupled to phosphorylation and the synthesis of energy-rich phosphate bonds. It would, therefore, not be unreasonable to consider whether the compound involved in the production of chromosome aberrations by EOC is adenosine triphosphate (ATP), or some similar compound containing energy-rich phosphate bonds. This possibility is supported by the fact that the radiomimetic effect of EOC is suppressed by DNP, which is known to uncouple phosphorylation from oxidation, whereas *ortho*-nitrophenol, which is inactive as an uncoupling agent, has no influence on the effect of EOC.

It is therefore suggested as a working hypothesis that ATP or a similar compound is involved in the production of chromosome aberrations by EOC and that oxygen is necessary for the effect because, in its absence, the formation of energy-rich phosphate is greatly reduced.

An obvious way to test the validity of this hypothesis seemed to be the carrying out of anaerobic EOC-treatments in the presence of ATP. In fact, such a treatment was performed, however, without obtaining any effect of ATP on the frequency of chromosome aberrations produced by EOC. (The concentration of ATP in the treatment solution was 10^{-3} M.) However, as pointed out by BULLOUGH (1952): »It seems experimentally impossible to introduce into a cell extra quantities of energy-rich phosphorylated compounds, since these compounds do not readily pass through a cell wall». Thus, the hypothesis suggested above is not invalidated by the negative result of the EOC-ATP-experiment. If the hypothesis should be correct, the question arises how ATP is involved. A possibility which should be considered is that it furnishes the energy necessary for the production of chromosome aberrations, and thus that structural chromosome changes can arise as a result of an ATP-reaction.

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SUMMARY

The influence of oxygen on the production of chromosome aberrations by *X-rays*, *8-ethoxycaffeine* (EOC), and *nitrogen mustard* has been studied with root tips of *Vicia faba* as experimental material. The main results so far obtained are as follows:

The radiomimetic effect of *nitrogen mustard* is not dependent on the oxygen tension during treatment.

On the other hand, oxygen influences the production of chromosome aberrations by *EOC* and *X-rays* in a very similar way, with the exception, however, that *EOC* in contrast to *X-rays* produces practically no effect in the absence of oxygen (Fig. 3). The effect of *EOC* is also reduced by pre-treatment exposures to anaerobic conditions, whereas that by *X-rays*, according to GILES and RILEY, is not.

When present during treatment, sodium azide, which inhibits the enzyme cytochrome oxidase and thereby respiration, suppresses the radiomimetic effect of *EOC* as completely as do anaerobic conditions. In contrast, the chromosome damage produced by *X-rays* is practically unaffected by sodium azide. These facts indicate that the oxygen effect operates by the way of the respiratory chain in the case of *EOC*, but not in the case of *X-rays*.

Furthermore, the effect of *EOC* is suppressed by 2.4-dinitrophenol, which is known to uncouple phosphorylation from oxidation.

It is suggested as a working hypothesis that adenosine triphosphate or a similar compound is involved in the production of chromosome aberrations by *EOC*, and that oxygen is of significance because, in its absence, the formation of energy-rich phosphate is strongly reduced.

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A NEW DIPLOID VARIETY OF POA ALPINA WITH TWO ACCESSORY CHROMOSOMES AT MEIOSIS

By *ARNE MÜNTZING* and *AXEL NYGREN*
INSTITUTE OF GENETICS, LUND, AND ROYAL AGRICULTURAL COLLEGE, UPPSALA, SWEDEN

I. INTRODUCTION

THE grass species *Poa alpina* L. is generally apomictic and composed of biotypes with chromosome numbers ranging from 30 to 50 (*cf.* MÜNTZING, 1954 b). Most of these numbers are aneuploid and in each geographic region special numbers may prevail. In a sexual strain from Switzerland MÜNTZING (1932, 1940) found the chromosome number to be oscillating but not lower than 21. In 1940, SOKOLOVSKAYA and STRELKOVA reported the finding of diploid *alpina* types with $2n=14$ from the Caucasus. In 1942, CHRISTOFF found that a *Poa alpina* type studied by him was also diploid and sexual, the sexuality being demonstrated by embryological investigations. This strain was of unknown origin but derived from a seed sample obtained from the Botanical Garden of Innsbruck. MÜNTZING received this strain from Dr. CHRISTOFF and discovered (1946) that some additional chromosomes were present at meiosis though not more than 14 chromosomes were to be found in the root tips. A detailed study of this strain was undertaken (MÜNTZING, 1948, 1954 a; HÅKANSSON, 1948, 1954) which in the main revealed that from 2 to 8 somewhat smaller accessory chromosomes were present at meiosis in every plant of the strain. These chromosomes are eliminated from all lateral parts of the plants (secondary roots and leaves) and are only retained in the panicles, at least in the pollen mother cells and embryo sac mother cells. In 1952 another diploid *Poa alpina* from the Tatra mountains in Poland was described by SKALINSKA, this material, however, being viviparous and not accessible to studies of meiosis.

In August 1953 one of us (NYGREN) made a field trip in Engadin, southern Switzerland, together with Professor WALO KOCH of Zürich in order to collect different *Poa* species of the *Oreinos* group. At Ardez a delicate *Poa alpina* was found which proved to belong to the variety

xerophila BRAUN-BLANQUET. Chromosome counts in root tips of 20 plants collected at this locality revealed that the strain in question was diploid, having $2n = 14$. It shows some morphological similarities with the one derived from the Botanial Garden of Innsbruck, and now it is fairly clear that the seed sample from Innsbruck represented native material. Earlier it was suspected that this strain was derived from Russia, a diploid *Poa alpina* being known from Caucasus, and the Swiss *alpina* types studied at that time all having fairly high chromosome numbers.

In June 1954 NYGREN definitely observed that the new strain from Ardez just as the Innsbruck strain had supernumerary chromosomes at meiosis. However, in the Ardez strain only *two* extra chromosomes were present. In a preliminary cytological study, based on aceto-orcein squashes, NYGREN observed some irregularities in the meiotic pairing and distribution of the extra chromosomes. An analysis of two loculi from different plants gave the result that in the first plant 14 p.m.c. out of 22 had 8 bivalents, the configuration $7_{II}+2_{I}$ occurring in the remaining eight cells. In the other loculus four cells had 8_{II} and five $7_{II}+2_{I}$. NYGREN's technical assistant carried out somatic counts in root tips of 112 plants raised from a mixed seed sample of the Ardez population. 105 plants were found to have $2n=14$, whereas in seven plants from 1 to 3 extra chromosomes seemed to be present. MÜNTZING had the opportunity of studying slides of 5 of these apparently deviating plants but found in all cases $2n=14$, the discrepancy between the counts probably being caused by the presence of a sometimes rather attenuated achromatic region in one of the chromosome pairs (*cf.* MÜNTZING, 1948, p. 56). Thus, the chromosome counts undertaken by NYGREN in the Ardez strain showed that $2n=14$ is the chromosome number regularly occurring in the root tips.

In the autumn of 1953 MÜNTZING had the opportunity of raising cultures of the Ardez strain, and in May 1954 fixations of mitosis and meiosis were carried out. A technical assistant made somatic counts in 47 individuals, 40 of which were found to have $2n=14$, whereas in 7 plants additional chromosomes were more or less clearly observed at least in some root tips or in some cells. MÜNTZING then made a detailed study of the somatic chromosome number in these 7 plants as well as in 7 other plants. Meiosis was also studied in this material, useful slides being obtained from 12 individuals. The root tips were fixed in chrome-acetic-formalin, the spikelets in Benda. Sections of root tips as well as of spikelets were stained with crystal violet.

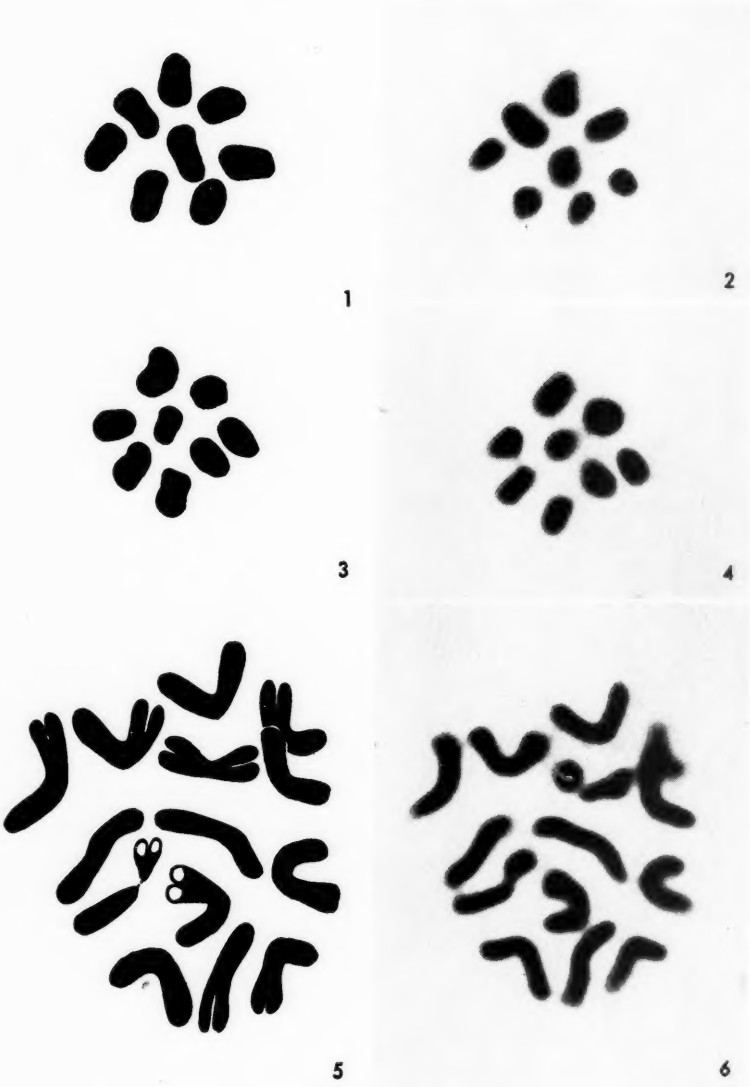


Plate I. Different chromosome numbers at mitosis and meiosis in the Ardez strain of *Poa alpina*. — Figs. 1 and 3, early I-A in polar view, the two plates of the same cell separately drawn, each containing 8 chromosomes; Figs. 2 and 4, photomicrographs of the same plates (Fig. 2 corresponding to Fig. 1, and Fig. 4 to Fig. 3); Fig. 5, somatic plate from root tip with $2n=14$; Fig. 6, photo of the same plate, the chromosome at 2 o'clock not quite in focus.

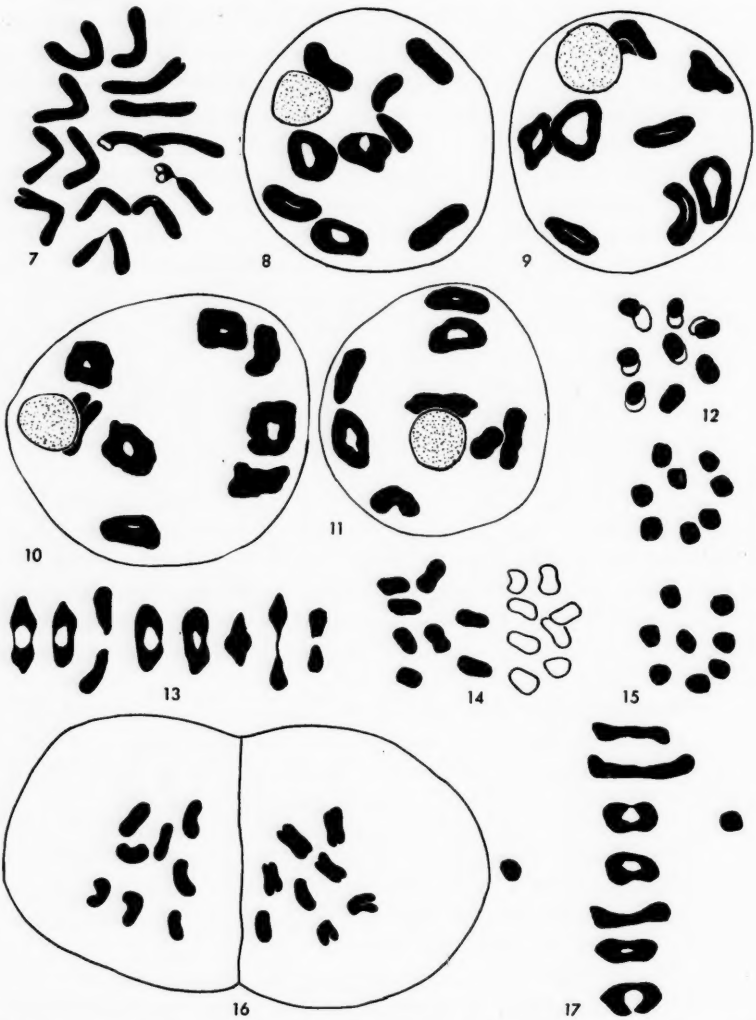
II. TYPICAL CHROMOSOME BEHAVIOUR

Of the 12 plants of the Ardez strain in which mitosis as well as meiosis was studied 7 represented a random sample. In all these plants as well as in two other ones, erroneously suspected of being deviating, the chromosomes behaved in a similar way. This typical behaviour, characterized by the presence of 14 chromosomes in the root tips and 16 chromosomes at meiosis is illustrated in Figs. 1—22. The essential point may be seen in Figs. 1—6 (Plate I). Figs. 1 and 3 represent the two corresponding plates of an especially good pollen mother cell at early first anaphase in polar view. The plates are separately drawn, and in Figs. 2 and 4 we have the corresponding photomicrographs, showing that the presence of 8 chromosomes in each group is indisputable. It is also of special interest that there are no obious size differences between these chromosomes and neither any observable differences in stainability or degree of stickiness. Nevertheless, one of these pairs is regularly eliminated from the somatic tissues or at any rate from the root tips. Fig. 5 shows a somatic plate of the same plant, in which there are clearly 14 chromosomes, and Fig. 6 represents a photo of the same plate.

Fig. 7 shows a somatic plate with $2n=14$ from another plant, the chromosomes in this case being somewhat less contracted than in Fig. 5. The two members of the chromosome pair with a median, almost achromatic region may easily be distinguished, the chromosome at 6 o'clock being especially typical.

The presence of an extra chromosome pair at meiosis is most easily seen at diakinesis. Figs. 8—11 show 4 p.m.c. at this stage from 4 different plants. In most cases the bivalents are closed rings of approximately the same size, none of them presenting any unusual features. Figs. 12 and 13 represent first metaphase groups in polar view and in side view (separately drawn), showing the normal behaviour with 8 bivalents of similar size. Figs. 14—15 show normal cells at I-A with 8 chromosomes in each group, and in Fig. 16 there is a cell at second metaphase, again showing the normal distribution 8—8.

Figs. 17—22 represent slight irregularities in otherwise normal plants involving the occurrence of non-conjunction in one chromosome pair. In Fig. 17 there are $7_{II}+2_{I}$, both univalents being situated outside the plate. In Fig. 18, showing the same configuration, one of the univalents (to the left) is situated in the equator, among the bivalents, is somewhat stretched and evidently orientated in the spindle. In the I-M group represented by Fig. 19 this is true of both univalents (Nos. 1 and 3 from the left).



Figs. 7—17. Typical cytology in the Ardez strain of *Poa alpina*. — Fig. 7, somatic plate, $2n=14$; Figs. 8—11, diakinesis with 8_{II} from four different plants; Fig. 12, I-M in polar view, 8_{II}; Fig. 13, I-M in side view (separately drawn); 8_{II}; Fig. 14, I-A in side view, distribution 8—8; Fig. 15, I-A in polar view, the two plates separately drawn, distribution 8—8; Fig. 16, II-M, 8 chromosomes in each plate; Fig. 17, I-M in side view (separately drawn), $7_{II}+2_{I}$.

The univalents (presumably the equatorial ones especially) may divide at I-A as is evident from Figs. 20—21, showing the distributions 7—2/2—8 and 8—9, respectively. In Fig. 22 the univalents are still undivided, the distribution being 7—2—7.

The following data are available with regard to the frequency of such deviations from perfectly normal meiosis:

In four plants a total of 237 cells at first metaphases were observed, 197 of them being without univalents, 40 having 2 univalents. According to these values, the percentage of regular I-M groups without univalents was 83,1. In five plants a total of 149 p. m. c. at *first anaphase* were examined, 131 being regular and 18 with lagging chromosomes. This corresponds to a percentage of 87,9 regular I-A groups. If only those data are included in which both stages were studied in the same plants, the corresponding values will be 85,3 for 187 I-M groups and 86,9 for 99 cells at I-A. The slightly higher values at I-A is in agreement with the observed fact that some univalents pass to the poles undivided.

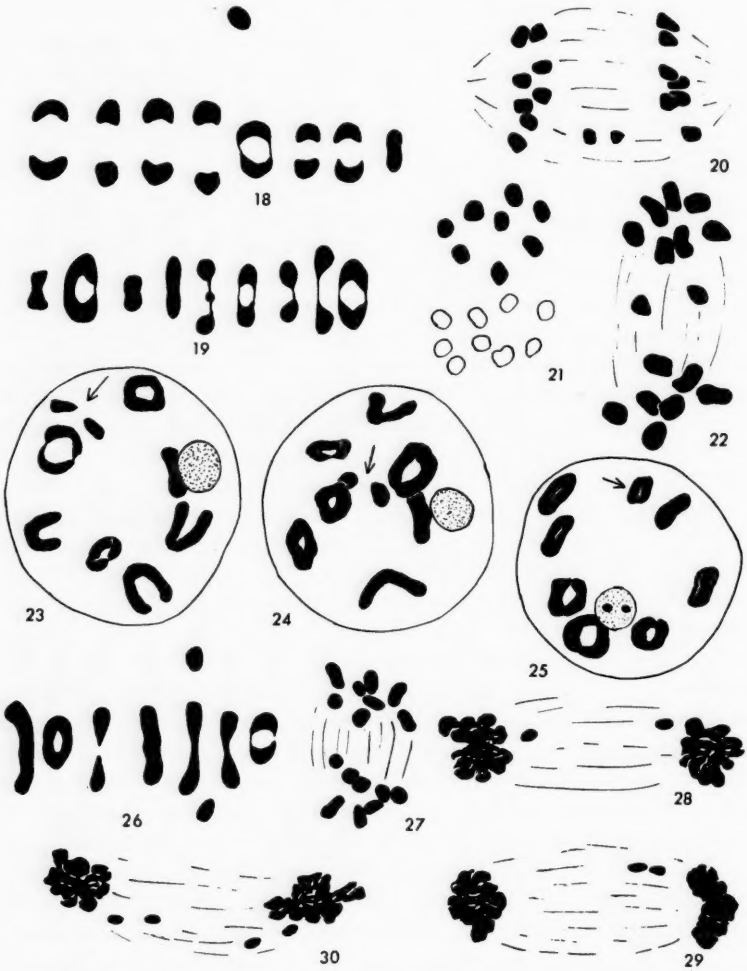
In five plants the types and frequencies of chromosome distribution at first anaphase was recorded. Of a total of 32 cells, in which both anaphase groups and eventual chromosomes lagging between the groups could be counted, 23 showed the normal distribution 8—8, the remaining nine cells representing the distributions 8—9, 8—2/2—7, 7—9, 7—2—7, 8—1—7 and 9—9. In 38 other cells only one of the anaphase groups could be counted, the chromosome number in this plate being 8 in 36 cases and 9 in 2 cases. Combining the two categories (cells in which both groups could be counted and cells in which only one group was clear) a total of 102 I-A groups was observed. Eighty-eight of these had 8 chromosomes, which represents a percentage of 86,3. This value closely corresponds to the frequency of regular I-A cells without lagging chromosomes. As mentioned above, this value was 87,9.

Though not very comprehensive the data just given are sufficient to demonstrate that in the majority of cells meiosis is perfectly regular. However, slight irregularities do occur in about 15 per cent of the p. m. c.

III. SOME DEVIATING PLANTS

I. A plant with smaller B-chromosomes also present in the root tips

Studies of meiosis in *Plant 1—40* revealed that one of the eight bivalents in this individual is definitely smaller than the other ones. This is evident from Figs. 23 to 25, representing p. m. c. at diakinesis. In these

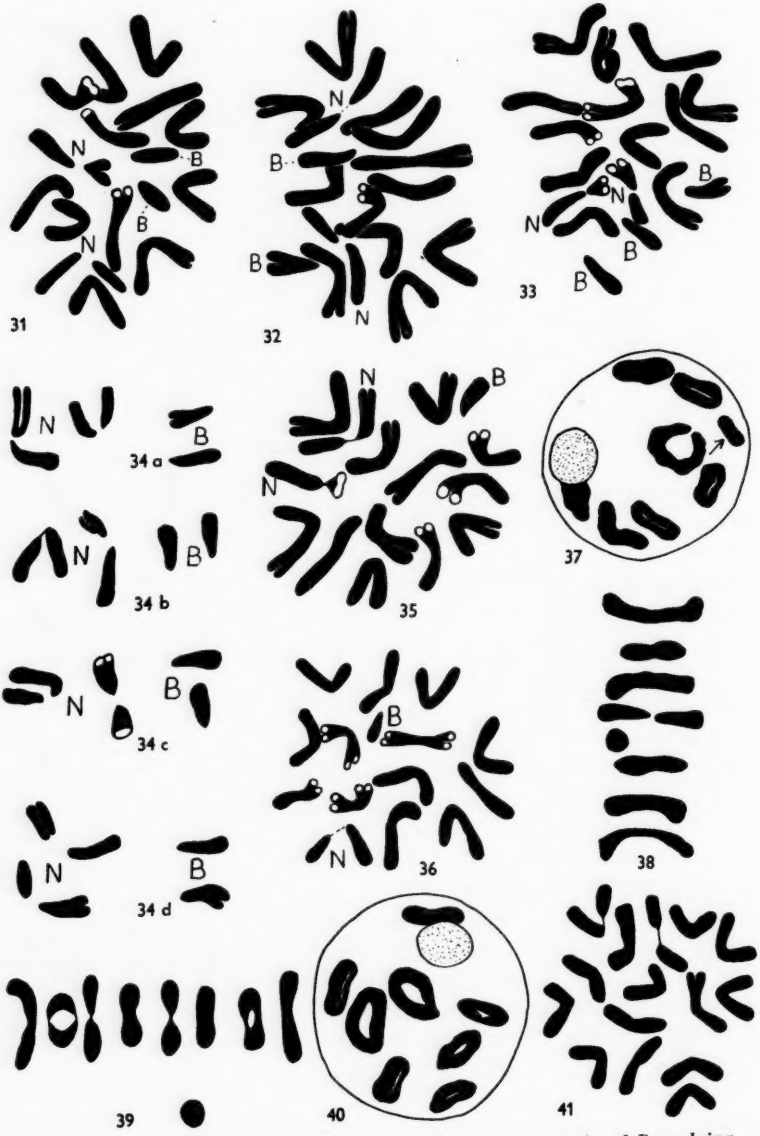


Figs. 18—30, meiosis in the Ardez strain of *Poa alpina* (continued). — Fig. 18, I-M (separately drawn), $7_{II}+2_{I}$, one of the univalents in the equator; Fig. 19, $7_{II}+2_{I}$ (separately drawn), both univalents in the equator; Fig. 20, I-A in side view, distribution 8—2/2—7; Fig. 21, I-A, polar view, distribution 8—9 (one univalent has divided); Fig. 22, I-A, side view, distribution 7—2—7. — Figs. 23—30, meiosis in a deviating plant with one smaller bivalent. Figs. 23—25, diakinesis with 8_{II} but one bivalent (indicated with an arrow) clearly smaller; Fig. 26, I-M, $7_{II}+2_{I}$; Fig. 27, I-A, distribution 8—8; Figs. 28—30, irregularities at late I-A; Fig. 28, lagging of 2 chromosomes; Fig. 29, division of one lagging chromosome indicated; Fig. 30, division of 2 lagging chromosomes.

cells the deviating bivalent is indicated by an arrow. It is rod-shaped in Figs. 23 and 24, and apparently a closed ring in Fig. 25. In the first two cells the connection between the two members of this bivalent is not visible but the shape and position of these chromosomes certainly indicate that they are really united. However, at first metaphase of this plant the presence of two univalents (Fig. 26) was much more frequent than in the normal plants, having 8 bivalents of approximately the same size. This fact as well as the relatively small size of the univalents (Fig. 26) certainly indicates that it is the small bivalent which is responsible for all or most of the non-conjunction occurring at I-M. Of a total of 100 p.m.c. observed 56 were regular while 44 showed the presence of two univalents. As mentioned above (p. 409), the percentage of regular I-M groups in four normal plants was on an average 83,1 per cent, the individual average values being 93,7, 81,7, 76,0 and 75,0. These values are based on a total of 237 cells, 197 of which had only regular bivalents and 40 two univalents. A comparison between this ratio 197 : 40 and the ratio 56 : 44 observed in the deviating plant under discussion gives a χ^2 of 27,7. As this value corresponds to a P smaller than 0,001 the difference is quite significant.

First anaphase in Plant 1—40 was predominantly regular (Fig. 27) but in several cells division of univalents could be observed. Fig. 28 seems to represent two lagging undivided univalents, Fig. 29 division of one univalent and Fig. 30 division of two univalents.

A study of mitosis in the root tips of Plant 1—40 turned out to be particularly interesting. In contrast to the »normal» plants, in which all the eight bivalents are of about the same size, Plant 1—40, having a small bivalent at meiosis, did not show any difference in chromosome number between somatic cells and pollen mother cells. Thus, *the two small chromosomes are also present in the root tips.* This is evident from Figs. 31 and 32, showing 12 ordinary chromosomes, 2 presumably nucleolar chromosomes (N) with a median achromatic region and 2 smaller chromosomes (B) which must correspond to the small bivalent observed at meiosis. The appearance of these last-mentioned chromosomes indicates that the centromere is terminal or almost terminal. This is best seen in Fig. 32 whereas in Fig. 31 they are too contracted to indicate a definite position of the centromere. The B-chromosomes present in Fig. 33, another somatic plate from the same plant, again demonstrate a terminal or subterminal position of the centromere. In this case there are 3 additional chromosomes of this kind and, thus, their number is variable. As a rule, however, the number is 2, this being



Figs. 31—41, cytology of some deviating plants of the Ardez strain of *Poa alpina*. — Figs. 31—34, somatic chromosomes in the plant with a small additional bivalent at meiosis (Figs. 23—30). Figs. 31—32, somatic plates with $2n=14+2$ smaller chromo-

found to be true in 12 out of 14 plates studied in this plant. Of the two deviating plates one had 3 B-chromosomes, the other one 1 B-chromosome.

Accurate somatic counts could only be made in plates in which both N-chromosomes could also be identified, the arms of the N-chromosomes sometimes being similar in size and appearance to the B-chromosomes. Thus, in Fig. 34 the N- and B-chromosomes of four different plates are separately drawn in order to prove that the B-chromosomes actually are special supernumerary chromosomes and not apparently free arms of the N-chromosomes. In these chromosomes one of the arms is somewhat longer than the other arm. The B-chromosomes represented in Fig. 34 again give evidence of the terminal or subterminal position of the centromere.

2. Plants with occasional small B-chromosomes at mitosis

In *Plant 1—15* a total of 32 somatic plates were studied, 30 of which followed the general rule, *i. e.* having 14 chromosomes in spite of the fact that 16 chromosomes were present at meiosis. In 2 plates, however, a single supernumerary chromosome was present. One such plate is represented in Fig. 35. It is rather obvious that with regard to size as well as centromere position the chromosome denoted with B in this plate is identical or very similar to the B-chromosome in Plant 1—40.

Another similar case was met with in *Plant 1—45*. Here a total of 13 somatic plates were studied, 11 of which were normal with $2n=14$, whereas 2 plates were deviating, one having one extra chromosome, the other two extra chromosomes. In both these plates the extra chromosomes were smaller than the other chromosomes and probably of the same type as in the previous plant, 1—15.

3. Cases representing chimaerical constitutions

In some plants differences in chromosome constitution between different parts of the same individual were found to occur. In *Plant 1—32* this was true with regard to the root tips. A total of 25 somatic plates in these root tips were examined. Two of these roots had only the normal

somes; Fig. 33, another plate with 3 B-chromosomes; Fig. 34, 2 B- and 2 N-chromosomes from each of four different somatic plates (a, b, c, d). Figs. 35—36, somatic plates with $2n=14+1$ B-chromosome from otherwise normal plants; Figs. 37—38, diakinesis and I-M (separately drawn) from sector with $7_{II}+1_I$; Fig. 39, I-M (separately drawn) with $8_{II}+1_I$ in a chimaerical plant; Fig. 40, diakinesis with 8_{II} from the same plant; Fig. 41, somatic plate of a trisomic plant ($2n=15$).

number 14 (8 and 7 plates examined, respectively) whereas in the third root most of the plates contained an extra chromosome of the kind illustrated in Fig. 36. This chromosome (denoted »B») is again smaller than the other chromosomes and its centromere must be terminal or subterminal. In Fig. 36 only one of the N-chromosomes may be distinguished. Nevertheless, it is excluded that the B-chromosome represents one of the arms of the other N-chromosome even though it corresponds in size to the short arm of the N-chromosome. Of 10 plates examined in this root tip 7 contained one extra B-chromosome, whereas 3 had only the 14 normal chromosomes.

In *Plant 1—20* a chimaerical structure was evident at meiosis. In a slide containing diakinesis and first metaphase stages most of the p.m.c. had 8 bivalents, but in a few special anther loculi the configuration was $7_{II}+1_{I}$, one chromosome being lost. This is evident from Fig. 37, showing diakinesis (univalent indicated by an arrow) and Fig. 38 showing first metaphase.

Plant 1—42 seems to represent a case of the opposite type, one supernumerary univalent being present in addition to the 8 bivalents. Fig. 39 shows a first metaphase of this kind with $8_{II}+1_{I}$. A total of 21 p.m.c. of this type was observed, these p.m.c. being mixed with 74 p.m.c. showing no univalents and 5 p.m.c. showing 2 univalents. For comparison Fig. 40 shows a normal diakinesis with 8_{II} of the same plant. With crystal violet this extra chromosome stained just as well as the regular chromosomes. As it was also rather uniform in size it does not seem probable that it could be, for instance, a nucleolus persisting to first metaphase in some of the cells. It was most probably a true chromosome, but its occurrence in mixture with normal p.m.c. is difficult to explain.

4. A trisomic plant

One plant, 1—19, had 15 chromosomes in the root tips and in this case the additional chromosome did not differ in size from the other chromosomes. From the somatic plate represented in Fig. 41, it is also rather clear that the plant is not trisomic for the N-chromosome, only two chromosomes of this kind being clearly visible. Unfortunately, in contrast to all the 46 sister plants this individual did not survive the winter, and thus meiosis could not be studied. However, its poor vigour lends further support to the view that this plant represented a true trisomic individual in which the extra chromosome belonged to the »A»-chromosomes which are not eliminated in the somatic tissues.

IV. SUMMARY OF THE SOMATIC COUNTS UNDERTAKEN IN TYPICAL AND DEVIATING PLANTS OF THE ARDEZ STRAIN

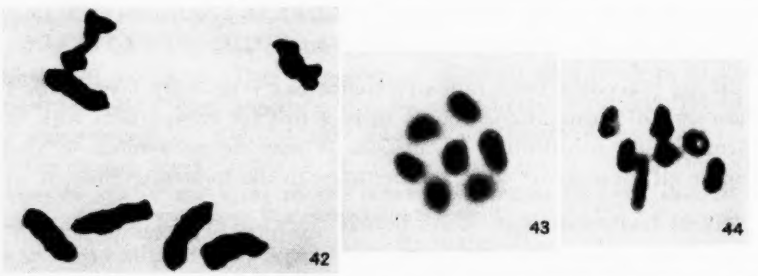
In the preceding paragraphs reference has repeatedly been made to the result of chromosome counts in root tips for comparison with the chromosomal constitution at meiosis. It may be convenient to summarize all the somatic counts undertaken in the following table:

Plant number	Number of somatic plates with chromosome numbers				
	14	15	14+1B	14+2B	14+3B
1—15	30		2		
—19		20			
—20	15				
—21	15				
—22	7				
—25	15				
—31	21				
—32 (root 1)	8				
» (» 2)	3		7		
» (» 3)	7				
—36	15				
—39	16				
—40			1	12	1
—42	18				
—45	11		1	1	
»Nygren 1»	12				

It should be observed that of the 14 plants listed above only 7 represent a random sample of the strain. In all these plants the characteristic conditions of the strain were found, the root tips only containing plates with $2n=14$ and the p.m.c. regularly having 8 bivalents. The seven other plants listed in the table were selected because the preliminary analysis of our technical assistant had indicated the occurrence of deviating chromosome numbers in the root tips. This was indeed verified for five of these plants, whereas in the remaining two individuals only regular plates with $2n=14$ could be found.

V. PRELIMINARY INFORMATION CONCERNING THE CYTOLOGY OF SOME OTHER DIPLOID STRAINS RELATED TO THE POA ALPINA GROUP

Through the courtesy of Dr. RECHINGER, Vienna, material has been obtained of *Poa badensis* KOCK, collected at the original locality near Vienna, Austria. A few plants were obtained which are morpholog-



Figs. 42—44, meiosis in *Poa badensis*. Fig. 42, late diplotene with 7_{II}; Figs. 43—44, I-M with 7_{II} in polar view and side view, respectively.

ically rather similar to the diploid strains of *Poa alpina* previously studied. Somatic counts showed that also *badensis* is diploid, having $2n=14$ in the root tips. The characteristic N-chromosomes with a median achromatic zone are also present in *badensis*. Meiosis was studied in two individuals, and in both plants there were clearly 7 bivalents at meiosis. Fig. 42 represents a p.m.c. at late diplotene, in Fig. 43 there is a first metaphase in polar view and in Fig. 44 a I-M group in side view. Fig. 43 may be compared with Figs. 1—4 of the Ardez strain in order to reveal an important distinction. *Though both strains have 14 chromosomes in the root tips only the Ardez strain has an extra bivalent at meiosis.* Further studies of *Poa badensis* may of course reveal the existence of plants with supernumerary chromosomes at meiosis, but the important point is that *badensis* plants without such chromosomes do occur. As the first plants studied have not been selected in any way it seems likely that in *Poa badensis* the occurrence of 7 bivalents at meiosis is typical. More extensive studies will show whether plants with accessory chromosomes may be found in a minority of the plants of this strain.

Finally, it may be mentioned that three diploid strains of *Poa* related to *alpina* but also reminiscent of *Poa laxa* were recently collected by Professor WALO KOCH in Hautes-Alpes, Eastern France. In one of them which may provisionally be called *Poa Molineri* (=*concinna*) preliminary meiotic observations were made by NYGREN. As in *badensis*, 7 bivalents were observed at diakinesis and late diplotene, indicating that accessory chromosomes are absent also from this diploid *Poa* type.

VI. DISCUSSION

The discovery of new diploid strains belonging or closely related to the collective species *Poa alpina* is of considerable interest with regard

to the origin of the apomictic strains which now dominate the species. At least some of the diploids are sexual and may represent progenitors of the apomicts which have more or less high and aneuploid chromosome numbers. The systematic position and relationship of these diploid strains may be dealt with in detail later on. Already now, however, it may be mentioned that, according to data obtained in 1954, the new Ardez strain just as the Innsbruck strain is self-sterile, as a rule no seeds being obtained in the isolation bags. Cross-pollination between the strains, on the contrary, gave plenty of seeds which germinated well and will probably give rise to F_1 hybrids. Crosses between the Ardez strain and *Poa badensis* were carried out in this summer (1955) and also in this hybrid combination some degree of seed setting was obtained. That these seeds are the result of cross-pollination is again indicated by the fact that very few seeds were obtained in the isolation bags, in *badensis* as well as in the Ardez strain.

Of *Poa badensis* only a few plants have so far been available, but of the Ardez strain rather large progenies have been raised by NYGREN and MÜNTZING. These progenies are fairly uniform but do not show the strict homogeneity characteristic of apomictic biotypes. They also comprise a low proportion of clearly deviating and rather aberrant plants with poor vigour, some of them also showing chlorophyll deficiencies. The Innsbruck strain, studied by MÜNTZING, is much more variable in morphology as well as vigour, and it remains to find out the cause of this obvious difference in degree of variability. One possible cause may be that in the Innsbruck strain the number of accessory chromosomes is oscillating between 2 and 8 (MÜNTZING, 1946, 1948, 1954; HÅKANSSON, 1954), whereas in the Ardez strain the chromosomal situation is much more balanced, almost all plants having 8 bivalents at meiosis.

In spite of this regularity the Ardez strain is most unusual by having a lower chromosome number in the root tips than at meiosis. In the typical cases (Plate I), which are quite predominant, the somatic plates in the root tips contain only 14 chromosomes and not 16 as should have been expected from the presence of 8 bivalents at meiosis. In this respect the Ardez strain is similar to the Innsbruck strain in which the somatic chromosome number is also 14 (in root tips as well as leaf cells) in spite of the presence of 2 to 8 additional chromosomes at meiosis. Otherwise in plant material such conditions are very rare and previously known only for one single species of *Sorghum* (JANAKI-AMMAL, 1940; DARLINGTON and THOMAS, 1941). Such phenomena are better known from

various insects, such as species of *Sciara* and the gall-midges, *Cecidomyidae* (*cf.* WHITE, 1954, pp. 239—257).

As the Innsbruck and Ardez strains show characteristic similarities as well as dissimilarities with regard to their chromosome behaviour it may be convenient to specify this more clearly in the following way:

Similarities: Presence of special chromosomes at meiosis but not at mitosis. This occurs in *all* members of the strains.

Dissimilarities: In the Ardez strain there are always 2 extra chromosomes at meiosis, these chromosomes not clearly differing in size, stainability or other respects from the rest of the chromosome complement. In the Innsbruck strain the extra chromosomes may be distinguished from the normal chromosomes by a smaller size, and a tendency to stickiness. Moreover, they are variable in number, different plants of the strain having from 2 to 8 »germ line» chromosomes.

Especially in the Ardez strain the germ line chromosomes may be regarded as regular members of the chromosome complement of the strain, and it is probable that they are of value or even indispensable for the plant. At least in some individuals of the related *Poa badensis* the situation is entirely different, 14 chromosomes being present at meiosis as well as mitosis just as in any ordinary regular diploid. This offers an excellent opportunity of further analysis by raising hybrids between the strains. If these hybrids can be produced and are viable, they may be expected to have one single germ line chromosome. Consequently, in F_2 the number of such chromosomes should range from 0 to 2. Such a material as well as back-crosses between F_1 and the parent strains may give valuable information concerning the influence of the germ line chromosomes on vigour, fertility and morphology and may also shed some light on the mechanism of somatic elimination of these special chromosomes.

With regard to this elimination one plant of the Ardez strain was found to be especially interesting. In this plant the eighth bivalent was smaller than usual and could be clearly distinguished from the other bivalents at diakinesis. At first metaphase it showed non-conjunction in 44 per cent of the cells, this frequency being significantly higher than in plants in which the eighth bivalent was of normal size. In the plant with the small extra bivalent no somatic elimination of this chromosome type occurred, the root tips generally showing 2 smaller chromosomes in addition to the normal complement of 14 chromosomes. It is striking that the small extra chromosomes in this case seem to have a terminal position of the centromere. This indicates that this chromo-

some may be a product of misdivision. At any rate a fairly large part of this chromosome must have been lost, and at the same time the somatic elimination has been stopped. This certainly indicates that the factor or factors responsible for the somatic elimination were located in the chromosome segment which has disappeared.

In some other plants occasional somatic cells were seen in which an extra chromosome of a quite similar type was observed to occur. In one of these cases three root tips were observed, one of these tips showed the presence of one such chromosome in most of the plates, whereas it was not found at all in the other two root tips examined. In all these cases the same or almost the same chromosome type seems to be present. Presumably it has escaped somatic elimination owing to loss of a special arm or segment capable of inducing this elimination.

In some cases the plants had a chimaerical constitution with regard to the germ line chromosome. In one plant, for instance, there were $7_{II}+1_{I}$ in some anther loculi, though the majority of the loculi contained p. m. c. with 8_{II}. In another plant p. m. c. with the configuration $8_{II}+1_{I}$ were seen. As already mentioned, other plants were chimaerical with regard to the root tips. Further, in the plant regularly showing small extra chromosomes in the root tips the number of these chromosomes was not quite stable, a few cells containing 1 or 3 chromosomes of this kind instead of 2. All this indicates a weak but noticeable tendency to mitotic irregularity of the germ line chromosome and its derivatives.

It is rather obvious that the germ line chromosomes in the two diploid strains of *Poa alpina* belong to, or are closely related to, the so-called accessory chromosomes occurring in several other plant species (*cf.* MÜNTZING, 1954 a). Somatic elimination of accessory chromosomes also occurs in *Sorghum* (DARLINGTON and THOMAS, 1941) but a peculiarity restricted to the *Poa* strains is the occurrence of the special chromosomes in every plant of the strains. In the Innsbruck strain their number is still fluctuating, but in the Ardez strain they have become regular members of the chromosome complement save their still remaining peculiarity, the somatic elimination. This seems to be the almost final link in a rather continuous series of cases (*cf.* MÜNTZING, 1954 a) in which the other extreme is represented by such species as rye. In this species accessory chromosomes only occur in some populations and usually in rather low frequencies. Their genetical effects have only been found to be deleterious, and apparently they are only retained in the populations thanks to a strong tendency to numerical increase by non-disjunction, this process occurring on the male as well as the female

side. From such extreme cases there seem to be rather continuous transitions to material, such as *Centaurea scabiosa* (FRÖST, according to MÜNTZING, 1954 a), in which the B-chromosomes may have a slightly positive selective value, and thus are maintained in some plants of the species population in spite of the absence of a mechanism of numerical increase. The *Poa* strains seem to have proceeded still further, the accessory chromosomes here probably being indispensable for the plants and thus more or less clearly belonging to the normal chromosome complement.

SUMMARY

(1) The grass species *Poa alpina* L. is generally apomictic and composed of biotypes with more or less high, usually aneuploid chromosome numbers. One diploid sexual strain from Innsbruck and one viviparous diploid from Poland are known previously. A new sexual diploid *alpina* belonging to the variety *xerophila* BRAUN-BLANQUET was discovered at Ardez, Switzerland. All these diploids have 14 chromosomes in the root tips.

(2) Just as the Innsbruck strain the Ardez strain has supernumerary chromosomes at meiosis which are not present in the root tips. Whereas the number of such chromosomes is oscillating between 2 and 8 in the Innsbruck strain it is regularly 2 in the Ardez strain. These 2 chromosomes show regular pairing at meiosis and form an extra bivalent in 85 per cent of the cells. This bivalent cannot be distinguished from the seven other normal bivalents. In the Innsbruck strain, on the contrary, the extra chromosomes are smaller and more sticky than the normal chromosomes.

(3) Some deviating plants in the Ardez strain were observed, the most interesting one having the two extra »B»-chromosomes smaller than the sister plants. In this case they paired less well at meiosis than in the normal sister plants having larger B-chromosomes. The small B-chromosomes were not eliminated from the root tips and seem to have a terminal centromere. They may be products of misdivision, the factor or factors responsible for somatic elimination being located in the segment or chromosome arm which had been lost. Identical or similar chromosomes were also observed in some roots or root sectors of otherwise normal plants with large B-chromosomes at meiosis.

(4) A certain degree of mitotic irregularity of the B-chromosomes is indicated by cases representing chimaerical constitutions, in which the number of B's was slightly higher or lower than normal.

(5) *Poa badensis* KOCK is another diploid *Poa* belonging to the *alpina* complex. The few plants examined had 14 chromosomes in the root tips and 7 bivalents at meiosis as in an ordinary diploid. Crosses between *badensis* and the Ardez strain (with 8 bivalents at meiosis) have been undertaken. Some other diploid strains of *Poa* were found in Hautes-Alpes, Eastern France. Their systematical position is not yet clear but they seem to be closely related to *Poa alpina.*

(6) The »germ line» chromosomes in diploid *Poa alpina* are compared with accessory chromosomes in other plant species.

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EXPERIMENTAL AND CYTOLOGICAL STUDIES ON PLANT SPECIES

III. PLANTAGO CORONOPUS AND ALLIED SPECIES

By *TYGE W. BÖCHER, KAI LARSEN* and *KNUD RAHN*
BOTANICAL LABORATORY, UNIVERSITY OF COPENHAGEN, DENMARK

I. INTRODUCTION

IN a previous paper (BÖCHER, LARSEN and RAHN, 1953) it was shown that *Plantago coronopus* embraces a number of races which differ in size and production of matter as well as duration of life. Between several diploid strains ($2n=10$) a single aneuploid strain ($2n=11$) and a hexaploid plant ($2n=30$) were found. The chromosomal unstability in the *P. coronopus* complex had already been emphasized by GUINOCHET and GORENFLOT (1952), who found three diploid and four tetraploid strains in North Africa. In the present paper it has been tried through cytological investigations and experimental cultivations to attain to a better understanding of the relations between the species of the *P. coronopus* group. The variation within *P. coronopus* along the West coast of Europe is further elucidated on the basis of many cultures particularly from Denmark, Eire, and Portugal. We hope later to be able to render an account of the results of some crossings between species and races belonging to the *P. coronopus* group.

We are indebted to Drs. A. R. PINTO DA SILVA and BENTO RAINHA, Sacavem, G. RODIO, Catania, A. FAHN, Jerusalem, G. A. H. ALSTON, London, and M. LANGE, Copenhagen, for several collections of seeds from various countries. Financial support has been obtained from the Carlsberg Foundation.

II. CYTOLOGICAL OBSERVATIONS

Chromosome number. — The material comprises 75 chromosome countings made mainly in root-tip mitoses. The fixative used was MÜNTZING's modification of NAVASHIN—KARPECHENKO's fluid. The sec-

tions were stained in Feulgen or gentian violet. The following chromosome numbers were found (for origin of material, see Tables 1—4):

P. coronopus L.	$2n=10$	(48 strains)
» » »	» =11	(4 »)
» » »	» =20	(a large sector in a root of a plant with $2n=10$, see Fig. 2 *a—b*)
» » »	» =30	(a single plant from Portugal (No. 2800), see Fig. 4 in the previous paper)
» » »	» =31	(a single plant from Portugal (No. 3378), see Fig. 2 *v*)
» *macrorhiza* POIR.	» =10	(2 strains)
» » ssp. *occidentalis* PILG.	» =10	(4 »)
» *commutata* GUSS.	» =20	(5 » ; two mentioned in Table 4, three from the Dalmatian coast to be cultivated later)
» *serraria* L.	» =10	(3 strains)
» » »	» =10+1 B	(3 strains)
» » »	» =10+3 B	(1 strain, see Fig. 2 *n—o, s—u*)
» » »	» =20+2 B	(a single plant of No. 3674, Fig. 4)
» *crassifolia* FORSK.	» =20	(1 strain, Fig. 2 *p*)

According to MCCULLAGH (1934), *P. crassifolia* has $2n=24$. In view of the great possibility of confusion with *P. maritima* this number may be erroneous. In the *P. maritima* group the numbers $2n=12$ and 24 occur; cp. MCCULLAGH (*l. c.*) and GREGOR (1939).

The geographical distribution of the chromosome numbers appears from Fig. 1. On the map are also the countings mentioned by GUINOCHET and GORENFLOT (with the exception of »Mokta-Douz») as well as the diploid *P. coronopus* from Portugal mentioned by RODRIGUES (1953). Evidently the polyploids only occur in the South, and the aneuploids seem so far to be restricted to the Atlantic parts of Europe from Jersey in the Channel to Algarve in Portugal. As in the case of *Kohlrauschia prolifera* only the diploids have been able to force their way to Northern Europe.

Size and morphology of somatic chromosomes. — *Plantago coronopus* and the closely related *P. macrorhiza* and *P. commutata* are clearly distinct from *P. serraria* and *P. crassifolia* by the size of the chromosomes (Fig. 2). Furthermore there are differences in the idiograms. *P. coronopus* has a pair of very long chromosomes which have submedian centromeres, and *P. serraria* is characterized by its very long SAT-chromosomes. The two very long chromosomes are also present

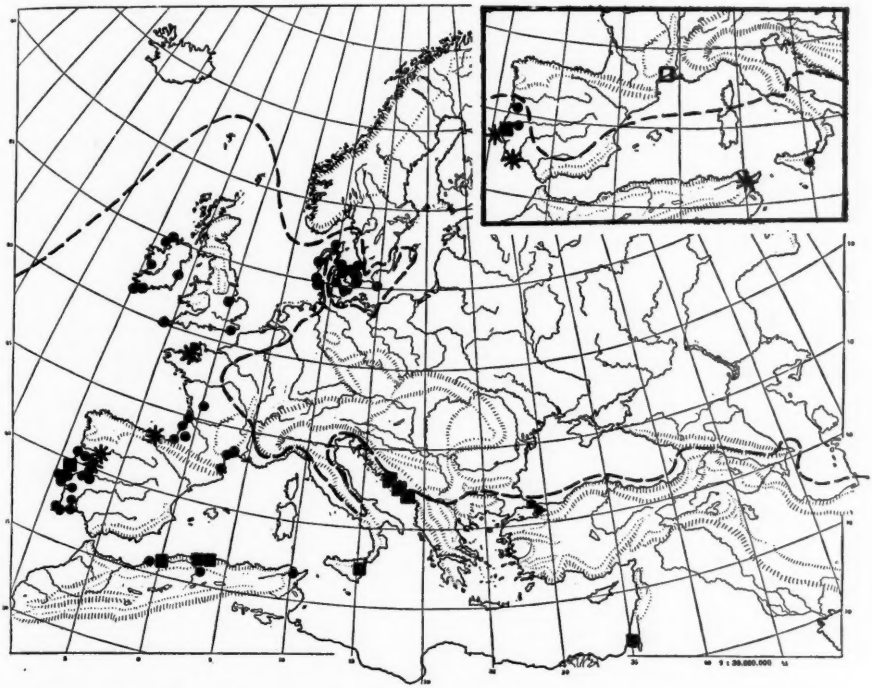

Fig. 1. Large map: Distribution of the *Plantago coronopus* complex (*P. coronopus*, *P. macrorhiza*, and *P. commutata*) in Europe. Northern limit of the complex indicated by a dotted line. Solid dots: diploids; asterisks: trisomic plants; quadrates: polyploids. — Small map: Distribution of *P. serraria* (northern limit indicated); dots: diploids; asterisks: diploids with 1—3 B-chromosomes; quadrate: tetraploid with 2 B-chromosomes. The quadrate near Montpellier is the station of the tetraploid *P. crassifolia*.

in *P. macrorhiza* (Fig. 2 *f*), and in the tetraploid *P. commutata* four long chromosomes are seen (Fig. 2 *q*). The cytological difference between *P. serraria* and *P. coronopus* is further stressed through the behaviour of the supernumerary chromosomes. In *P. coronopus* the shortest chromosome with submedian centromere is tripled. In the figures this chromosome is indicated with a β. From slides stained in Feulgen it appears that the supernumerary chromosome is never heterochromatic. Thus, the aneuploids of *P. coronopus* are trisomic. In *P. serraria*, on the other hand, the supernumerary chromosomes are smaller than any of the other chromosomes. They are subterminally

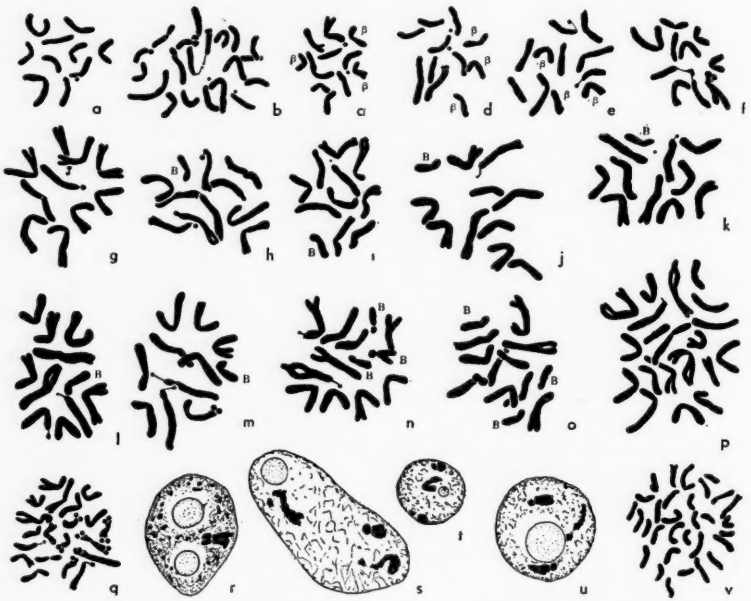

Fig. 2. Somatic cells of *P. coronopus* (*a—e, v*), *P. macrorhiza* ssp. *occidentalis* (*f*), *P. serraria* (*g—o, r—u*), *P. crassifolia* (*p*), and *P. commutata* (*q*). — *a—b*: No. 3372, two mitoses from the same root tip with $2n=10$ and 20; *c*: No. 1351 and *d—e*: No. 3377, both trisomic with $2n=11$; *f*: No. 3382 ($2n=10$); *g*: No. 3302 ($2n=10$); *h—k*: No. 3385 ($2n=10+1$ B); *l*: No. 3072 and *m*: No. 3674, also with $2n=10+1$ B; *n—o*: No. 3290 with $2n=10+3$ B; *r*: resting nucleus of No. 3674 with one heterochromatic B-chromosome; *s—u*: resting nuclei of No. 3290 with three heterochromatic B-chromosomes (*s* young root hair cell, *t* dermatogen cell, *u* and *r* periblem cells); *p*: No. 3071 and *q*: No. 3291, both with $2n=20$; note the difference in chromosome size; *v*: No. 3378 with $2n=31$. — ×2100.

constricted, and in resting nuclei stained in Feulgen they are present as overstained bodies which frequently have the shape of a mitotic chromosome with subterminal constriction (Fig. 2 *r—u*) and which even may appear as if the chromatids already had split (Fig. 2 *r*). In this species the supernumerary chromosomes thus have all the characteristics of B-chromosomes or accessory chromosomes. In Fig. 2 they are indicated by the letter B. Their shape and relative size correspond to those of the standard B-chromosome in rye (MÜNTZING, 1944; LIMA-DE-FARIA, 1948), but not to the standard accessory in *Festuca pratensis*, which has a median centromere (BOSEMARK, 1954).

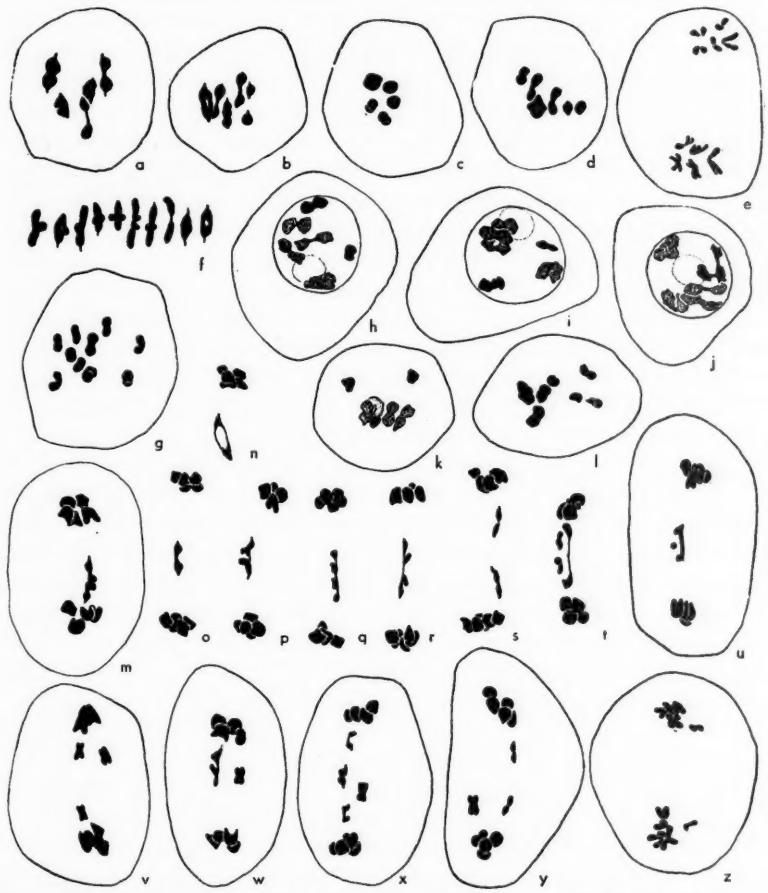

Fig. 3. Meiosis in *P. macrorhiza* (*a*), *P. macrorhiza* ssp. *occidentalis* (*e*), *P. commutata* (*f*—*g*), and *P. coronopus* (*b*—*d*, *h*—*z*). — *a*: No. 3289, *b*—*d*: No. 3755, *e*: No. 3375, *f*—*g*: No. 3291, *h*—*z*: No. 3377 (trisomic). *d*: 4 II and 2 I; *h*—*i*: 5 II and 1 I; *j*: 4 II and 1 III; *k*—*l* (early metaphase I): 4 II and 3 I; *m*—*y*: ana- or telophase I showing the behaviour of the β-bivalent or the three β-univalents; in Fig. *u* the lagging β-bivalent forms a bridge and an acentric fragment; *z*: metaphase II (side view) with two isolated laggards outside the plates. — ×2100.

There are two SAT-chromosomes. Only in the tetraploid sector in the root of *P. coronopus* No. 3372 four chromosomes carried trabants (Fig. 2 *b*). In the tetraploid *P. commutata* only two SAT-chromosomes were seen. The same was the case in the tetraploid *P. crassifolia*

(Fig. 2 *p*), and there two of the four very short chromosomes had a peculiar small dilatation at the centromere (Fig. 2 *p* above on the right and central in the plate).

Meiosis in diploid P. coronopus and P. macrorhiza. — This was perfectly regular in the strains from Northern Europe of *P. coronopus* as well as in *P. macrorhiza*, which was studied in material from Tunisia (Fig. 3 *a*) and Portugal (Fig. 3 *e*). The two very long chromosomes form a large bivalent, which frequently is a ring with two terminalized chiasmata and is somewhat lagging. The shortest chromosomes, on the other hand, form a pair which in many PMCs at metaphase I are separated before the rest (Fig. 3 *c*). In one of the southern strains lack of pairing of the shortest chromosomes occurred in a few cells (Fig. 3 *d*), a fact which contributes much to the understanding of the origin of the trisomic plants which may be formed as results of non-disjunction of the two short chromosomes.

Meiosis in trisomic P. coronopus. — During the prophase the three short β-chromosomes behave as the other chromosomes. At diplotene and diakinesis they almost always form one univalent and one pair (Fig. 3 *h*—*i*). Only a single cell among very many which were carefully studied had all three chromosomes joined in a trivalent (Fig. 3 *j*). Such cells must be very rare as also during metaphase I no cases of trivalent formation were seen. At this stage the rule was one univalent, but in not a very few cells all three β-chromosomes were unpaired (*e. g.*, Fig. 3 *k*—*l*).

The anaphase of the first division was very interesting. It appeared that the β-pair is very frequently lagging, whereas the supernumerary β-chromosome may either go undivided to one of the poles or it may be left as a laggard which split precociously. The lagging β-bivalent, as a rule, has one localized chiasma. In one case only two terminalized chiasmata were seen (Fig. 3 *n*). One cell contained a lagging bridge with an acentric fragment (Fig. 3 *u*), but it is not unlikely that Fig. 3 *t* shows a case with another inversion bridge. The fragment, however, was not visible or was extremely small, appearing as a minute body in the middle of the bridge. Corresponding to such cells which during metaphase had three univalent β-chromosomes, some cells at anaphase I had three lagging β-chromosomes, which split simultaneously (Fig. 3 *v*) or successively (Fig. 3 *x*).

The localization of the chiasma in the β-bivalent is interesting in view of the strictly terminalized chiasmata in the other bivalents. The defective pairing and the occurrence of inversions indicate that in this

case the localization may be the result of structural changes (cp. discussion in BÖCHER, 1945). The univalent chromosomes are always stretched along the spindle fibres. In other cases, *e. g.*, in monosomic *Triticum*, they lie across the spindle fibres (SANCHEZ-MONGE, 1950). In one case (Fig. 3 *x*) one of the univalents resembled a small bivalent in structure.

The distribution of the chromosomes is most frequently $4+1\,\beta$—$4+2\,\beta$, but if one, two, or all the three β-chromosomes are lagging and divide precociously, other distributions will occur. Lagging, dividing β-chromosomes are very rarely included in the interphase nuclei, but they form small independent nuclei close to the large ones. At the second telophase an isolated chromosome may frequently be observed near two of the nuclei. Obviously the laggard is included in the pollen grain as no dwarf pollen is formed. On the other hand, some of the grains are larger and others smaller than the majority and among the latter some are shrivelled. In other trisomic species (*e. g.*, rye, TAKAGI, 1935; *Polygonatum sibiricum*, SUOMALAINEN, 1947) the extra chromosome behaves in a similar manner. It may either go undivided to one pole at the first division and undergo a regular division at the second, or it may already split during the first anaphase and be found as a laggard during the second anaphase.

As the stainability of the three β-chromosomes does not differ from that of the other chromosomes they must be regarded as normal A-chromosomes. On the other hand, the facts that all the three are sometimes unpaired and that the β-bivalent is a laggard with localized chiasmata as well as the occasional formation of inversion bridges strongly indicate that they are structurally abnormal. The frequent occurrence of one pair and one univalent may further show that one of the β-chromosomes is particularly deviating. Perhaps bridges are only formed when this most deviating chromosome forms a pair together with one of the two others. It does not seem unlikely that such a supernumerary chromosome, which owing to its structure only rarely can pair, may be transitional to what has been called B-chromosomes. It may represent a stage in the chromosomal evolution leading to true B-chromosomes. In this connection it should be remembered that a pair of B-chromosomes in *Phleum phleoides* can form an inversion bridge and a minute acentric fragment and that structural re-arrangements of the A-chromosomes in this species accompany the occurrence of B-chromosomes (BÖCHER, 1950). Something quite similar is the case in *Clarkia* (LEWIS, 1951).

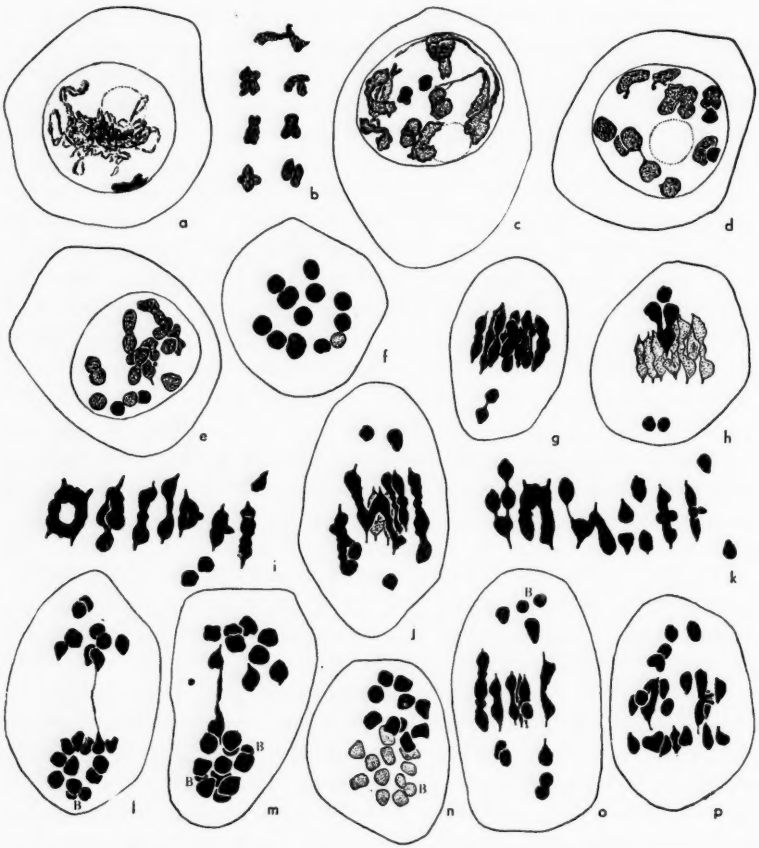

Fig. 4. Meiosis in tetraploid *P. serraria* (No. 3674) with two B-chromosomes. — *a*: pachytene with B-bivalent; *b*: B-bivalents from different pachytene cells; *c*: late diplotene; *d*—*e*: diakinesis (in *c*—*e* the B-chromosomes are black, in Fig. *e* there are 2 III, 5 II, 4 I, and 2 B); *f*—*k*: metaphase I (in *f* 10 bivalents and distribution of the B-chromosomes to both poles); *g*: B-bivalent outside the metaphase plane; *h*: a trivalent, a univalent and the two B-chromosomes outside the plane; *i*—*j*: 4 IV, 1 III, 4 II, 1 I, and 2 B; *k*: 3 IV, 4 II, and 2 B; *l*—*m*: anaphase I with inversion bridges and distribution 9—13; *n*: distribution 10—12; *o*: metaphase I [1 III, 6 II (one separated), 5 I (one dividing), and 2 B]; *p*: early anaphase I with two lagging bivalents, two univalents, and two B-chromosomes. — ×2100.

Meiosis in tetraploid P. commutata. — This was studied in material from Sicily and Jerusalem. In both cases meiosis was quite regular No trivalents or quadrivalents were observed in many PMCs at diakinesis and metaphase I (Fig. 3 *f*—*g*). This fact contributes to the establishment of *P. commutata* as a separate species (cp. p. 449).

Meiosis in tetraploid P. serraria with two B-chromosomes. — One of the plants of Cult. No. 3674, in which $2n=10$ (+1 B) was counted in root tips (Fig. 2 *n*—*o*), was fixed for the purpose of studying meiosis. The plant in question, however, although apparently not different from the other plants, appeared to be tetraploid with two B-chromosomes. The large number of multivalents makes it very probable that the plant in question is an autotetraploid.

Already during pachytene the B-chromosomes are easily detected as they are very precocious and form a pair which is separated from the rest and placed peripherically in the nucleus (Fig. 4 *a*). The condensation corresponds to that at late diplotene in the other chromosomes. Fig. 4 *b* shows the pair from different cells at pachytene. Below on the right the two B-chromosomes, however, are unpaired or they are already separated. It is most probable that they undergo a precocious separation as at later stages of the prophase they are generally separated although they are never removed much from one another until metaphase I. At this stage they may sometimes occur together with the bivalents on the equatorial plane, but as a rule they are placed between this plane and the poles either both on the same side or one on each side and not infrequently very close to the poles. In a single cell a delayed separation of the B-chromosomes was observed (Fig. 4 *g*). A counting of 50 PMCs in side view at metaphase I gave as result that in 33 of the cells both B-chromosomes were placed on the same side, in 16 cells they lie on both sides, and in one cell only they were included among the bivalents.

As a consequence of the early separation and the lack of orientation during metaphase I the B-chromosomes in this tetraploid plant will most frequently go to the same pole. This mechanism results in the formation of pollen grains with an increased number of B-chromosomes. A normal distribution during anaphase I, therefore, is 10 A—10 A+2 B (see, *e. g.*, Fig. 4 *n*).

In many cases, however, the distribution is much more unequal owing to the frequency of trivalents (Fig. 4 *h*—*j*) or the occurrence of unpaired chromosomes (Fig. 4 *e*, perhaps also Fig. 4 *o*—*p*, where, however, the early metaphase separation of some of the bivalents complicates the picture). A very frequent distribution is 9 A—11 A+2 B; it

is to be seen in the two cells with bridges (Fig. 4 *l*—*m*), but is without any connection with the occurrence of inversions. The interphase nuclei looked normal without isolated chromosomes. The tetrads were also regular, but in certain loculi about 10 per cent dwarf pollen were formed. In most anthers the size of the ripe pollen varies as much as might be expected if the chromosome number oscillates between 8 and 14.

Inversion bridges with acentrics are not common (Fig. 4 *m*). In some cases the fragment could not be detected or it might perhaps be absent (Fig. 4 *l*). Trivalents and quadrivalents were very frequent. The highest number of quadrivalents is 3 (Fig. 4 *k*).

The non-disjunction of the B-chromosomes in rye which was found during the pollen mitoses by MÜNTZING (1946) and at the first division in the embryo-sacs by HÅKANSSON (1948) made a study of the same mitoses in *P. serraria* desirable. But unfortunately our material only contained pollen with one or two resting nuclei and no embryo-sac mitoses. The above-mentioned behaviour of the two B-chromosomes in tetraploid *P. serraria*, however, shows that another type of non-disjunction occurs in this case already during the first meiotic division resulting in a numerical increase in the B-chromosomes. We have not yet got material of meiosis and the pollen mitoses in the diploid strain with three B-chromosomes from Africa. In diploids, however, the numerical increase must be due to some kind of somatic non-disjunction.

It is a remarkable fact that two related species both may contain supernumeraries and that these in one of them are typical B-chromosomes, whereas in the other they may be A-chromosomes which are structurally deviating. It is not known whether the other strains with B-chromosomes in *P. serraria* are structural hybrids as is the case of Cult. No. 3674, but so far our material corroborates the idea that A-chromosomes through structural changes, misdivisions, and mutations may develop into B-chromosomes which at their final stage are unable to pair with the original chromosomes (cp. LEWIS, 1951, on *Clarkia elegans*).

III. CHROMOSOME NUMBER AND TAXONOMY

GUINOCHET and GORENFLOT (1952) refer their material to the taxonomical units proposed by PILGER (1937) and state that *P. coronopus* ssp. *cupani* is diploid, whereas *P. coronopus* ssp. *eucoronopus* and ssp. *commutata* are composed by diploids and tetraploids. One of the tetra-

ploid races of ssp. *commutata* formed a transition to *P. macrorhiza*. Experimental cultivations carried out later by GORENFLOT (1954 a and b) according to this author reveal that the finding of tetraploids has very much complicated the taxonomy within *P. coronopus*. Material of ssp. *eucoronopus* and ssp. *commutata*, one of them from the dunes and tetraploid, the other from the mountains and diploid, may resemble one another to such an extent that they make a taxonomical distinction difficult.

A taxonomical utilization of the chromosome numbers found by us is also rendered difficult. It is a fact that diploids, polyploids, and aneuploids may occur in the same population and that the chromosomal diversity is accompanied only by a very moderate morphological one. In the strain from Sacavem (No. 2800), mentioned in our previous paper, probably only the plant pictured was hexaploid. Other plants from the same culture but with slightly smaller anthers and flowers proved to be diploid. The tetraploid *P. serraria* also had comparatively large flowers and anthers, but it was otherwise a rather low plant with the same general appearance as the diploid plants. As was the case, *e. g.*, in the strain No. 3378, the population was a mixture of different chromosome races. In this connection No. 3372 is of special interest. There, one plant had in a root a large tetraploid sector, a fact which involves the possibility of similar sectors in the inflorescence. Obviously *P. serraria* and many southern populations of *P. coronopus* are cytologically unstable, the unstability including structural changes, the formation of supernumeraries, and doubling of the chromosome number. Therefore, one might get an impression that in the present complex of species it was impossible to find any closer connection between the chromosome number and the taxonomy. It would of course be possible to circumscribe the polyploids by using a number of small quantitative differences, but considering the wide-ranging polymorphy among the diploids and the tetraploids severally, polyplotypes of this kind would at most be evaluated as forms or perhaps varieties.

For a further discussion the material of tetraploids from the East Mediterranean area is of particular interest. At our disposal are strains from Sicily, the Dalmatian coast, and Israel. In spite of certain reciprocal divergencies these strains in our opinion deserve being referred to an independent species. The plants from Israel and Sicily are extremely early, of low growth and with almost linear leaves or leaves with a few short lobes on each side of the rhachis (Figs. 8—9). The scapes are short and frequently the spikes do not overtop the leaves. The spikes are dense

and the bracts short. The complete regularity of the meiosis and the lack of multivalents indicate that the tetraploid number in this case may be the result of amphidiploidy or that the two chromosome sets of an original autopolyploid have undergone many and important changes. In PILGER's paper such plants are referred to *P. coronopus* ssp. *commutata* PILGER with appurtenant dwarfish forms. No doubt an emendation of the tetraploid species is desirable, but it must for the present be postponed until more is known about the diploid material which GORENFLOT refers to ssp. *commutata*. In the present paper we have decided to term the species *P. commutata* GUSS., which must be its right name as GUSSONE (1832, 1842) just describes it from Sicily, from where we have material which is tetraploid.

The plants with B-chromosomes or the trisomic plants are not morphologically distinguishable.

IV. EXPERIMENTAL CULTIVATIONS

In the years 1950—1954 many strains from various countries have been cultivated. The seeds were sown in March or early in April and the young plants were planted on the experimental field in June. Measurements were undertaken at the end of the growing season in September. In the first four experiments (Exp. 1—4) in the summers of 1950—1953 all plants were grown in the field, but in 1954 they were divided into two batches one of which was kept in pots in the Botanical Gardens (Exp. 6) and the other planted in the field (Exp. 5). The six experiments are summarized in Tables 1—4.

As distinct from many other pauciennials in our experiments *P. coronopus* and allied species show no signs of inhibition of flowering (cp. BÖCHER, LARSEN and RAHN, 1955). The plants can always develop as summer-annuals, but in nature they are frequently winter-annuals.

In the tables the figures refer to average values of measurements of the following characters:

B: Largest diameter of the basal leaf rosette in cm.
L: » length of scapes (peduncles) and spikes in cm.
S: » » » spikes in cm.
R: » breadth of rhachis (in *P. serraria* and typical *P. macrorhiza* of the leaf) in cm.
W: Weight of dry matter per plant in g in September—October.

The strains are arranged according to size (in Tables 2—4, the size

TABLE 1. *Cultivations 1950—1952.*

Plantago coronopus.

Cult. No.	Origin and collector [1]	2n	Experiment	No. of plants	Characters; see p. 434					
					B	L	B×L: 100 [2]	S	L — S: S	R
1312	France: Les Landes 1 — B. and L.	10	1	17	10	10	1,0	2,1	3,8	0,1
2281	Denmark: Kregme — B. and L.	10	1	12	14	14	2,0	3,7	2,8	0,3
1320	France: St. Jean de Luz — B. and L.	10	1	11	15	19	2,8	5,8	2,3	0,4
2332	Denmark: Amager — B. and L.	10	1	11	19	18	3,5	5,0	2,6	0,4
1271	France: Les Landes 2 — B. and L.	10	1	16	22	20	4,3	5,5	2,6	0,3
2464	Portugal: Coimbra *	10	1	9	19	25	4,9	7,5	2,3	0,4
1288	France: Biscarosse — B. and L.	10	1	6	22	24	5,2	7,5	2,2	0,3
1351	Spain: Mioño — B. and L.	11	1	14	25	21	5,3	6,6	2,2	0,5
2521	France: Montpellier *		1	8	24	26	6,2	7,2	2,6	0,4
1735	» : Béziers, Roque Haute — B. and L.	10	1	13	27	31	8,4	9,0	2,4	0,5
1718	France: Argèles — B. and L.	10	1	9	32	36	11,4	10,6	2,4	0,3
2720	England: Lincolnshire — A.	10	2	19	10	9	0,9	1,7	4,3	0,2
2674	Denmark: Skansehage — B.	10	2	15	19	17	3,2	3,8	3,5	0,4
2640	» : Avernakø — B.	10	2	21	20	18	3,7	5,3	2,4	0,6
2707	» : Helgenæs — L.	10	2	20	25	21	5,3	7,2	1,9	0,7
2631	England: Folkestone — A.	10	2	18	32	26	8,4	8,7	2,0	0,8
2800	Portugal: Sacavem *	30 / 10	2	19	39	32	12,5	11,2	2,0	0,7
2998	Denmark: Rømø — L.	10	3	9	11	13	1,5	4,2	2,1	0,3
2851	» : Hammeren, Bornholm — B.	10	3	10	14	12	1,6	3,3	2,6	0,3
2863	» : Thorsminde — B.	10	3	10	15	16	2,3	4,9	2,3	0,3
2999	» : Lolland — B.	10	3	10	14	18	2,5	5,6	2,2	0,4
2756	England: Cornwall near Lizard Point — A.	10	3	11	23	17	3,9	5,6	2,0	0,4

[1] B.=T. W. BÖCHER.
L.=KAI LARSEN.
A.=Dr. G. A. H. ALSTON, London.
* Seeds from wild sources obtained through Botanical Gardens.

[2] B×L: 100 calculated on the basis of the original figures with decimals.

in Exp. 4), this being expressed through the rosette diameter and the length of scapes+spikes (size-index: B×L: 100). The ratio between the lengths of the scapes and the spikes (L—S: S) is taxonomically important and is given in a special column. In addition to the characters

TABLE 2. *Cultivations 1953—1954.*
Material of P. coronopus from Northern Europe.

Cult. No.	Origin and collector[1]	2n	Experiment	No. of plants	Characters; see p. 434						
					B	L	B×L:100	S	L−S:S	R	W
3227	Eire, Slieve League, alt. 450 m — B.	10	4	15	8	7	0,5	2,0	2,5	0,3	2
			5	12	8	6	0,4	1,4	3,3	0,3	3
			6	10	7	6	0,4	1,4	3,3	0,3	
3283	Jersey, Grosnez Point — B.	10	4	19	12	10	1,1	2,0	4,0	0,2	3
3163	Eire, Three Castle Hd. — B.	10	4	19	13	10	1,2	2,2	3,5	0,3	7
3216	» , Annagary, Donegal — B.	10	4	15	15	11	1,6	2,3	3,8	0,2	8
3166	» , Toormore Bay — B.	10	4	15	16	11	1,8	2,1	4,2	0,2	9
3200	» , Oranmore — B.	10	4	16	17	11	2,0	2,4	3,6	0,2	5
2863	Denmark, Thorsminde, Jutl. — B.	10	4	15	14	15	2,1	4,3	2,5	0,3	5
2851	» , Hammeren, Bornholm — B.	10	4	18	15	15	2,3	3,6	3,2	0,4	6
3122	Denmark, Sejerø — B	10	4	19	18	16	2,9	4,7	2,4	0,3	12
3133	Eire, Dun Laoghaire Hb. — B	10	4	19	20	14	2,9	4,5	2,1	0,3	16
3516	Sweden, Kullen — B.	10	5	15	20	15	3,0	3,9	2,8	0,4	11
			6	9	14	12	1,6	2,7	3,4	0,3	
3548	Denmark, Rubjerg Knude — B.	10	5	13	22	16	3,4	5,2	2,1	0,5	19
			6	6	12	11	1,3	3,0	2,7	0,4	
3286	Jersey, Ouens Bay — B.	11	4	19	23	16	3,6	4,7	2,4	0,3	18
3275	» , Bouley Bay — B.	11	4	15	22	17	3,8	4,8	2,5	0,3	19
3755	France, Périgeux *	10	5	12	24	20	5,0	6,3	2,2	0,4	15
			6	9	13	13	1,6	3,4	2,8	0,3	
3453	Denmark, Sønderho, Fanø-B. and L.	10	5	11	22	28	6,1	4,8	4,8	0,3	9
			6	8	17	16	2,7	3,7	3,3	0,3	

[1] B.=T. W. BÖCHER.
L.=KAI LARSEN.
* Seeds from wild sources obtained through Botanical Gardens.

mentioned in the tables the habit of the plants was noted, more particularly the direction of the primary and secondary leaves and the scapes. Finally, typical specimens of each strain were pressed with a view to studying the floral characters.

The weight of dry matter was studied in Experiments 4—5. In 1953 (Exp. 4) five plants which were estimated to be of average size were collected on July 3th, August 12th, and September 17th. In 1954 (Exp. 5) five such plants were collected on October 8th. After drying the plants were weighed.

TABLE 3. *Cultivations 1953—1954.*
Material of P. coronopus from Southern Europe.

Cult. No.	Origin and collector[1]	2*n*	Experiment	No. of plants	Characters; see p. 434						
					B	L	B×L : 100	S	L−S : S	R	W
3373	Portugal, Estramadura: Sintra — P.	10	4	15	23	18	4,0	4,6	2,9	0,7	14
			5	13	28	20	5,5	4,8	3,2	0,7	25
			6	10	17	15	2,4	3,4	3,4	0,4	
3380	» , Algarve: Olhão (Vulpinetum) — P.	10	4	15	20	21	4,4	9,2	1,3	0,6	24
			5	11	29	26	7,6	8,8	2,0	0,6	13
			6	10	16	21	3,3	5,5	2,8	0,5	
3376	Portugal, Alto Alentejo: Cumiada — P.	10	4	15	23	22	5,0	9,7	1,3	0,3	53
			5	15	35	30	10,2	10,8	1,8	0,6	31
			6	10	24	29	6,9	7,7	2,8	0,5	
3377	Portugal, Beira Alta: Famos de Algodres — P.	11	4	15	27	20	5,4	6,9	1,9	0,4	24
			5	13	32	24	7,8	7,6	2,2	0,6	30
			6	9	18	21	3,8	5,4	2,9	0,4	
3371	Portugal, Baixo Alentejo: Torrão 1 — P.		4	15	23	26	6,0	10,1	1,6	0,5	25
			5	14	36	31	11,2	11,7	1,6	0,8	31
			6	10	20	29	5,8	8,8	2,3	0,8	
3378	Portugal, Beira Baixa: Near S. Vicenta de Beira — P.	10 (31)	4	15	24	25	6,1	11,4	1,2	0,5	23
			5	14	36	29	10,6	12,6	1,3	0,9	38
			6	9	25	27	6,7	8,9	2,0	0,6	
3374	Portugal, Estramadura: Lisboa — P.	10	4	15	25	26	6,4	10,2	1,5	0,7	24
			5	13	35	34	11,9	11,5	2,0	0,8	36
			6	9	24	34	8,2	9,4	2,6	0,7	
3379	Portugal, Beira Baixa: Idanha a Nova — P.	10	4	15	24	28	6,5	12,1	1,3	0,5	29
			5	9	31	26	8,0	10,1	1,6	0,8	26
3381	Portugal, Beira Baixa: Castelo Branco — P.	10	4	15	26	26	6,7	10,4	1,5	0,4	56
			5	10	31	29	9,2	10,6	1,7	0,7	32
			6	10	19	23	4,4	7,7	2,0	0,6	
3304	Portugal, Sacavem *	10	4	18	29	31	8,8	10,8	1,9	0,7	34
3372	» , Baixo Alentejo: Torrão 2 — P.	10 (20)	4	15	32	28	9,1	11,7	1,4	0,9	52
			5	15	37	31	11,5	13,8	1,2	0,9	21
			6	10	19	23	4,4	8,0	1,9	0,6	
2800A	Portugal, Sacavem *	10	5	15	39	29	11,3	9,2	2,2	0,6	44
			6	10	29	31	8,9	7,2	3,3	0,4	
2800B	» , Sacavem *	10	5	14	42	36	14,9	12,0	2,0	0,5	54
			6	10	34	32	11,1	9,2	2,5	0,7	
3761	Bulgaria: Sofia *	10	5	15	30	28	8,2	10,2	1,7	0,5	15
			6	9	23	29	6,8	9,0	2,2	0,4	
3526	Turkey: Kisil-Adalar — M.	10	5	13	33	34	10,1	12,1	1,8	0,8	30
			6	8	26	38	9,9	10,4	2,7	0,9	
3528	» : Istanbul, Suliman Mosque — M.	10	5	9	36	33	11,9	10,8	2,1	0,8	44
			6	8	28	35	9,9	17,3	1,0	0,7	
3671	Tunisia: Ariana *	10	5	13	37	34	12,3	11,2	2,0	1,1	33
			6	9	23	34	7,7	7,3	3,7	0,9	

[1] P.=Drs. A. R. PINTO DA SILVA, and B. RAINHA, Sacavem, Portugal.
M.=Dr. MORTEN LANGE, Copenhagen.

TABLE 4. *Cultivations 1953—1954. Material of P. macrorhiza (3384—3073), P. commutata (3292—3291), and P. serraria (3385—3302).*

Cult. No.	Origin and collector [1]	2n	Experiment	No. of plants	Characters: see p. 434 B	L	B×L:100	S	L—S:S	R	W
3384	Portugal, Algarve: Cabo de S. Vicente — P.	10	4	16	16	15	2,5	5,4	1,8	0,5	16
			5	8	24	18	4,2	6,4	1,8	0,7	24
			6	4	18	17	3,0	3,9	3,4	0,6	
3383	Portugal, Algarve: Cabo de S. Vicente — P.	10	4	17	18	16	2,9	5,6	1,9	0,6	23
			5	12	27	18	5,0	5,9	2,1	0,8	21
			6	11	14	12	1,6	4,4	1,7	0,6	
3382	Portugal, Algarve: Cabo de S. Vicente — P.	10	4	15	18	17	3,0	6,7	1,5	0,6	21
			5	15	25	18	4,4	5,5	2,3	1,0	23
			6	10	21	15	3,1	4,9	2,1	0,6	
3375	Portugal, Estramadura: Praia des Marçás — P.	10	4	15	24	14	3,4	2,9	3,8	0,8	26
			5	11	31	19	5,9	4,9	2,9	0,7	
			6	7	14	9	1,3	2,8	2,2	0,6	
3289	Tunisia: Ariana *	10	4	20	29	23	6,7	8,1	1,8	1,1	
3073	France: Paris, Jardin des Plantes	10	4	8	35	38	13,4	12,9	1,9	1,2	
3292	Italy: Catania — R.	20	4	15	9	8	0,8	2,6	2,1	0,1	2
			5	10	14	10	1,3	3,5	1,9	0,2	3
			6	8	12	10	1,2	3,1	2,2	0,3	
3291	Israel: Jerusalem — F.	20	4	15	16	15	2,3	6,7	1,2	0,4	4
			5	13	13	12	1,5	4,8	1,5	0,3	3
			6	7	10	11	1,1	4,4	1,5	0,4	
3385	Portugal, Algarve: Pechão — P.	11 [2]	4	15	24	17	4,2	7,0	1,4	1,2	
3293	Italy: Catania — R.	10	4	11	25	21	5,1	6,8	2,2	1,7	
3290	Tunisia: Ariana *	13 [2]	4	20	26	20	5,2	8,9	1,2	1,6	
3302	Portugal: Sacavem *	10	4	6	28	22	6,1	9,8	1,2	1,8	

[1] P.=Drs. A. R. PINTO DA SILVA, and B. RAINHA, Sacavem, Portugal.
R.=Professor G. RODIO, Catania.
F.=Dr. A. FAHN, Jerusalem.

[2] 10+1 and 10+3 B chromosomes.

The material of *P. coronopus* is considered in Tables 1—3, that of the other species (but not *P. crassifolia*) in Table 4.

The figures only give information about differences in quantitative characters which are of less importance for a taxonomical treatment and which by comparison of the Experiments 4—6 appear to be much

influenced by changes in the environment. Experiments 4 and 5 were made two successive years on two different experimental fields. Experiment 6 was made in pots, as mentioned above. The soil was moister and the summer more rainy in Exp. 5 than in Exp. 4.

The figures are average values, and as no more than about 10—20 plants are available, no standard errors could be calculated. The ranges of the values measured are omitted for want of space. They are, however, mentioned in the text when comparisons are made.

The main results of the cultivations are considered in the next section where all important cytological and experimental data are synthesized in an experimental taxonomic treatment.

V. EXPERIMENTAL TAXONOMY

The monograph of the genus *Plantago* by PILGER (1937) has been very valuable as a basis of our studies. As in the case of *P. lanceolata* (cp. BÖCHER, 1943), it appeared that the units of orthodox taxonomy corresponded very well to certain genetical races or to ranges within continuous character gradients. On the other hand, the cytological and experimental results were thought in many cases to make a revaluation of the taxonomical rank of a taxon desirable.

The section Coronopus

PILGER divides this section into a *P. coronopus* group and a *P. maritima* group. These groups probably ought to be raised to forming independent sections, *viz.* a *Coronopus* section with 5 and a *Maritima* section with 6 as the basic chromosome number. Furthermore it is not excluded that the small Mediterranean section *Hymenopsyllium* might be included in the *Coronopus* section. According to MCCULLAGH (1934), two of its three species have $2n=10$ and the chromosome morphology corresponds to that in *P. coronopus*. Important accordances are further found in the morphology of the anthers and the corolla although the tube of the latter is pubescent in the *Coronopus* section and glabrous in the *Hymenopsyllium* section. The main difference between these sections may be found in the seeds which in *Coronopus* have a dimple whereas in *Hymenopsyllium* they are sulcate.

Within the *Coronopus* section two natural groups of species occur, one with small chromosomes centering around *P. coronopus* and another with larger chromosomes around *P. serraria*; cp. p. 450.

The Plantago coronopus group

Our material is referred to the three species *P. coronopus*, *P. macrorhiza*, and *P. commutata*. Among these, it seems to be most difficult to separate *P. macrorhiza* and *P. coronopus*, which are both diploids (the latter in the south with occasional polyploids or aneuploids) and which may be connected through intermediate forms the nature of which ought to be studied by crossings. According to PILGER, the transition is formed by *P. macrorhiza* ssp. *occidentalis* PILG. We have studied four cultures of this taxon from Portugal (Table 4). They did not, however, look like crossings but constituted a distinct race (see below). On the other hand, our material of *P. macrorhiza* obtained from Paris as seeds collected in nature (No. 3073) was intermediate between our large-growing Mediterranean races of *P. coronopus* and typical *P. macrorhiza*.

P. coronopus L.

All cultivated strains may be referred to ssp. *coronopus* (=*eucoronopus* PILG.), which by PILGER is divided into numerous varieties and forms. In our experience most of these varieties must be looked upon as ranges within a continuous very large and many-sided morphological variation. As appears from the experiments, this variation is accompanied by an equally large physiological one concerning earliness, duration of life, and responses to the habitat. It is easiest and safest to express the variation in terms of clines (cp. GREGOR, 1939).

Size and weight of dry matter. — Along the west coast of Europe the size and the production of dry matter increase considerably from the north to the south.

Extreme dwarf races are found in the northwest, in our material particularly from exposed rocks in Western Eire and Jersey, but according to herbarium studies they further occur in Scotland, in the Shetlands, and on Suderø among the Faroe Islands. In Denmark and Scania somewhat similar small plants were seen on wind-exposed places by the sea. In culture, however, they were all larger than those from Eire and Jersey (Fig. 5). In Exp. 4 (Table 2) the averages of the size indices of the smallest strains from the two areas are very different and the ranges do not overlap [No. 3227: 0,5 (0,2—0,8), No. 2863 2,1 (1,6—2,6)]. The three strains from exposed situations in the West (Nos. 3227, 3283, and 3163) have the smallest size indices in our material as a whole, thus indicating that a moderate increase in size can be demonstrated also in the direction from west to east in Northern Europe.

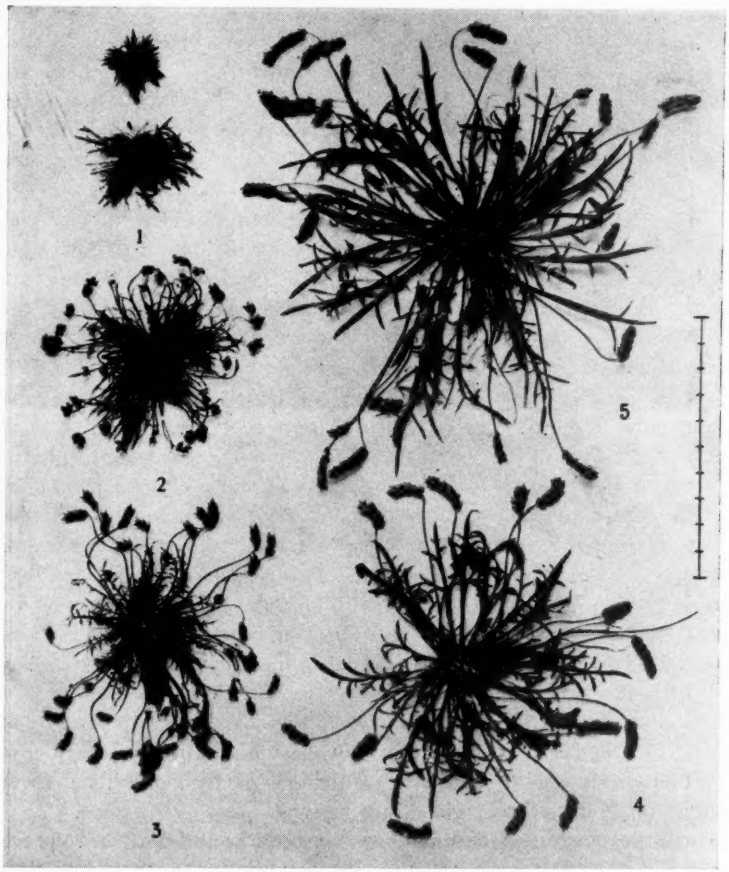

Fig. 5. *Plantago coronopus* from exposed cliffs or rocks in North-west Europe. — 1—2: No. 3227 from Slieve League, Donegal (1 collected in nature, 2 cultivated); 3: No. 3283 cultivated from Grosnez Point on Jersey; 4: No. 3548 cultivated from Rubjerg Knude; 5: No. 3516 cultivated from Kullen (rocks at the lighthouse). Scale 10 cm.

Undoubtedly extreme dwarfs occurring in nature in Denmark and Scania are to a higher extent modified by the environment [see, *e. g.*, the cultures from Rubjerg and Kullen (Table 4), both raised from seeds collected from very dwarfish plants]. Races of this kind which occur in a very special habitat constitute a typical ecotype in the sense of

TURESSON (1922). A closer study, however, discloses that this ecotype is not a discrete one, but is connected with other types through many intermediate ones. It is further evident that different dwarf races have developed in the two areas mentioned. The western dwarf type is smaller and has shorter and ciliate bracts whereas the bracts of the dwarfs from Denmark and Scania are glabrous as is generally the case in other strains from these countries. Also ecologically they may be different. Thus, the western dwarfs will grow high up in the mountains of Western Eire and have immigrated to the rough wind-swept »Ejder» of the Faroes (cp. OSTENFELD, 1908, pp. 930 and 934), whereas the Danish-Swedish populations have not produced races capable of invading the Norwegian west coast, which would seem to be fit for the western dwarf races (cp. Fig. 1). It is a question how great the differences between such populations must be before it becomes necessary to recognize the existence of two different ecotypes (cp. GREGOR and WATSON, 1954). In the case of *Plantago coronopus* it seems most natural to conceive one large polymorphic population with clinal variation and assume that different ranges within the clines (which frequenty have the same course) may be cut out at a rough estimate and be regarded as ecotypes (or topotypes). Thus, on the topocline for ciliation of the bracts running from Denmark to Portugal two different ecoclines can be recognized, one in the Northwest leading to an extremely dwarfish ecotype with ciliate bracts and another in South Scandinavia which may end with the low-growing race with glabrous bracts found, *e. g.*, on Bulbjerg and constituting another ecotype. Taxonomically, the dwarfs belong to f. *pygmaea* LGE., which, however, undoubtedly partly covers dwarf modifications of medium-sized races, partly genotypical dwarfs such as those described from Slieve League.

The largest average size indices are between 5,3 and 6,1 (No. 3453 from Fanø, Fig. 6) in Denmark and between 3,8 and 8,4 (No. 2631 from Folkstone) in the British Isles. In Spain and Portugal the smallest size index found in the experimental field is 4, but in many strains it is between 10 and 12 and it may reach 15. The northern limit for the tall-growing, long-spiked races with a size index of 10 or more runs from Southern France (*e. g.*, No. 1718, Table 1) to Turkey (Nos. 3526 and 3528, Table 3).

The weight of dry matter was supposed to rise constantly during the growing season. This also appeared to be the case of the southern races and particularly of the tall ones. In the period July 3th—August 12th the percentage increase for the southern races was 34 and in the period

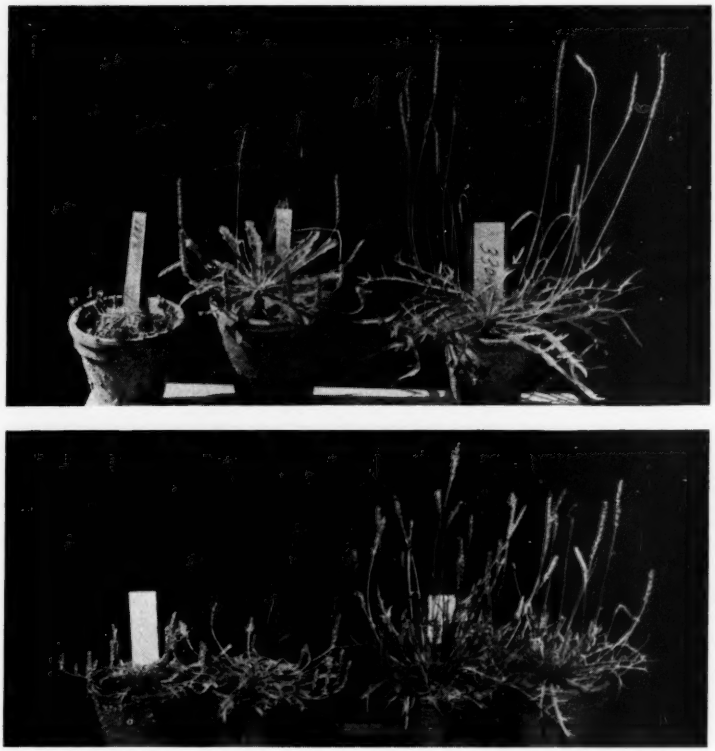

Fig. 6. Growth habit differences and size differences in *Plantago coronopus*. Above on the left dwarf race from Slieve League (No. 3382) compared with a tall race from Sacavem, Portugal (No. 3304), on the right. In the middle *P. macrorhiza* ssp. *occidentalis* (No. 3382) from Algarve. Below on the left two plants of the strain from Rubjerg Knude (No. 3548, exposed cliff by the North Sea), compared with two plants of No. 3453 from the dunes at Sønderho, Fanø, on the right. All plants raised from seeds collected in nature. Photographed on August 11th and 14th.

August 12th—Sept. 17th it rose to 136. The northern races, on the other hand, deviated by exhibiting a decrease of 20 per cent in the former period and in the latter an increase of 67 per cent. Among the northern strains the three dwarfish ones from Eire and Jersey had first a decrease of 43 per cent and later an increase of 34 per cent only. The plants of northern origin apparently did not stand the conditions in the experimental field in the driest part of the summer very well. This result agrees with the fact that *P. coronopus* in the north nearly always

grows near the sea on slopes or on hummocks in salt marshes where the humidity of the air may be relatively high and the temperature not very high. In the south it is frequent inland and grows there in gravel or among stones in very dry places. Thus, the northern and southern races seem to differ with regard to water-economy.

Leaf morphology. — In all the strains the leaves are distinctly pinnatifid, but they differ very much in size and shape of the pinnae and in the breadth of the rhachis (R in the tables). Several strains of southern origin have a comparatively broad rhachis (R) which is further expressed in the ratio B: 2 R. This ratio in Exp. 4 varies between 44 (No. 3200 from Eire) and 16 (No. 3373 from Portugal). In No. 3304 (Fig. 6) it is 20,5. Low values are also found in the small plants from exposed stations, a fact which mostly, however, is due to the shortness of their leaves. On the rocks of Hammeren (Bornholm) and on the isle of Rømø seeds were collected from plants on which the rhachis of the short leaves was very broad, the leaves at the same time being indistinctly pinnatifid. The plants raised from the seeds (Nos. 2851 and 2998), however, did not turn out to be less pinnatifid than usual and the rhachis was not particularly broad (R 0,3—0,4; B: 2 R 19 and 18), a fact which shows that these characters are much influenced by the environment. On the other hand, the above-mentioned increase in the breadth of the rhachis in a southerly direction is not a result of environmental changes, but may perhaps be explained as introgression from *P. macrorhiza.*

The variation in thickness and pubescence of the leaves is great, but at present we are unable to demonstrate any regular distribution of the characters in question. According to DODDS (1953), high degree of succulence in Britain may be confined to the south-west.

Growth habit. — The direction of the rosette leaves and of the scapes varies considerably. In the great majority of strains from the south the primary leaves are somewhat ascending and the secondary leaves are ascending or even almost erect. On the other hand, they are almost adpressed to the soil in the majority of plants of northern origin (Fig. 7). The angle of the scapes mostly follows and exceeds that of the leaves. In some southern plants they ascend steeply or are almost erect and in some of the dwarfs from the north they may be prostrate to adpressed and first arching just below the spike. As many northern plants come from comparatively humid habitats there may seem to be a parallelism with *P. maritima* in which, according to GREGOR (1938), the growth habit becomes progressively more erect as the habitat changes from

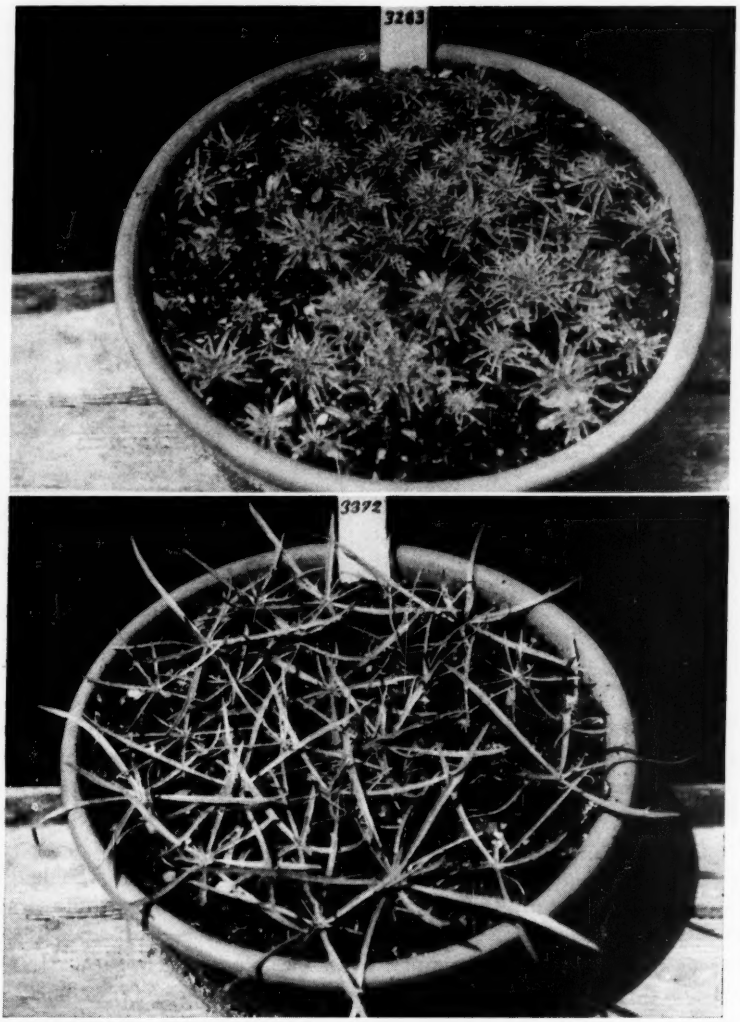

Fig. 7. Two extremes within *Plantago coronopus*; above from Grosnez Point (ocean bluff), below from Baixo Alentejo in Portugal. The seeds were sown late in March, the plants germinated in April and the photographs were taken on May 28th, 1953. Note the dense, adpressed rosettes with pinnatifid leaves of the Jersey plants and the open rosettes with slightly ascending, almost linear primary leaves in the Portuguese plants.

Fig. 8. *Plantago commutata*; young plants of the strain from Jerusalem photographed on May 28th, 1953; cp. Fig. 7. The plants are 8 weeks old and have already spikes (*e. g.*, at 2, 3, 5, and 9 o'clock; clearly visible at the arrow at 5'oclock). Note the succulent leaves with very short and few pinnae.

waterlogged to drained mud. Fig. 6 below shows that the two extremes both occur in Denmark. It is interesting to note that the plants with erect scapes originate from relatively dry soil in the dunes of Fanø in the North Sea. Those from Rubjerg Knude grew very exposed on the seaward slope, but the soil below was not sandy.

Scape, spike, and flowers. — The ratio scape length : spike length (L—S : S) is interesting. It is highest in the northern strains [average of all strains in Tables 2 and 3 is 3,1 and 2,1, respectively. In Exp. 4 it is 3,1 (range 2,1—4,2) in northern and 1,6 (1,2—2,9) in southern strains]. The character is of importance for the distinction between diploids and tetraploids in *P. media* (RAHN, 1954) and also of some importance for the distinction of *P. coronopus* from the tetraploid *P. commutata.* It is peculiar, however, that in *P. media* the tetraploids have long scapes, whereas *P. commutata* has comparatively short scapes (cp. p. 449).

The pubescence of the calyx and the bracts increases from the north to the south. Danish plants usually have glabrous bracts and in mat-

erial from Eire, England, and France the bracts are frequently sparsely ciliate along the margins. Most strains from the Mediterranean area and some from France and Jersey are densely ciliate and sometimes pubescent on the surface of the bracts. An increasing hair density on the bracts is generally accompanied by an increasing pubescence on the calyx and the corolla tube. The northern dwarfs from Eire and Jersey have rather short bracts and the same may be said about some of the tall strains from Portugal. In this character such plants approach *P. commutata*, but they are easily distinguished from this species through the laxness of the spike.

The differences in size between flowers of diploid and polyploid *P. coronopus* appear from Figs. 4 *a*—*e* in our previous paper (1953).

Duration of life. — *P. coronopus* is a typical pauciennial which after luxuriant flowering is inclined to die away, and thus may be annual, but it is also able to stand the winter and flower again next year. In some strains even three-year-flowering plants occurred. It is important that not a single plant in all our cultures failed to flower the first summer; thus, the species never behaves as a biennial spending the first year in a vegetative stage. In our previous paper (1953) it was mentioned that the faculty of wintering increases in a northerly direction. This result can be confirmed, but at the same time it must be admitted that many of the southern strains are capable of wintering when placed in a house which is kept free from frost and a few specimens may even sometimes stand the winter outdoors. In the house the plants of southern origin continued flowering during the winter. The main axis might grow constantly, but in some plants it formed a new rosette on top of the old one and this new, vernal rosette had narrow leaf segments and a narrow rhachis as compared with the old one.

P. macrorhiza POIR.

Our material clearly belongs to two different units which correspond to ssp. *macrorhiza* (=*eumacrorhiza* PILG.) and ssp. *occidentalis* PILG. (=*P. coronopus* var. *pseudomacrorhiza* COUT.). The strain from Tunisia (No. 3289, Table 4) is a typical ssp. *macrorhiza*; it is perennial when cultivated in a house without frost during winter and it has spatulate, glabrous, succulent and dentate leaves (Fig. 9). The other strains from Portugal mentioned in Table 4 are ssp. *occidentalis* and differ by the narrower middle part of the leaves and by having short lobes instead of teeth. They are further frequently annuals even when placed in a house during winter. Those from Algarve were very pubescent and with longer

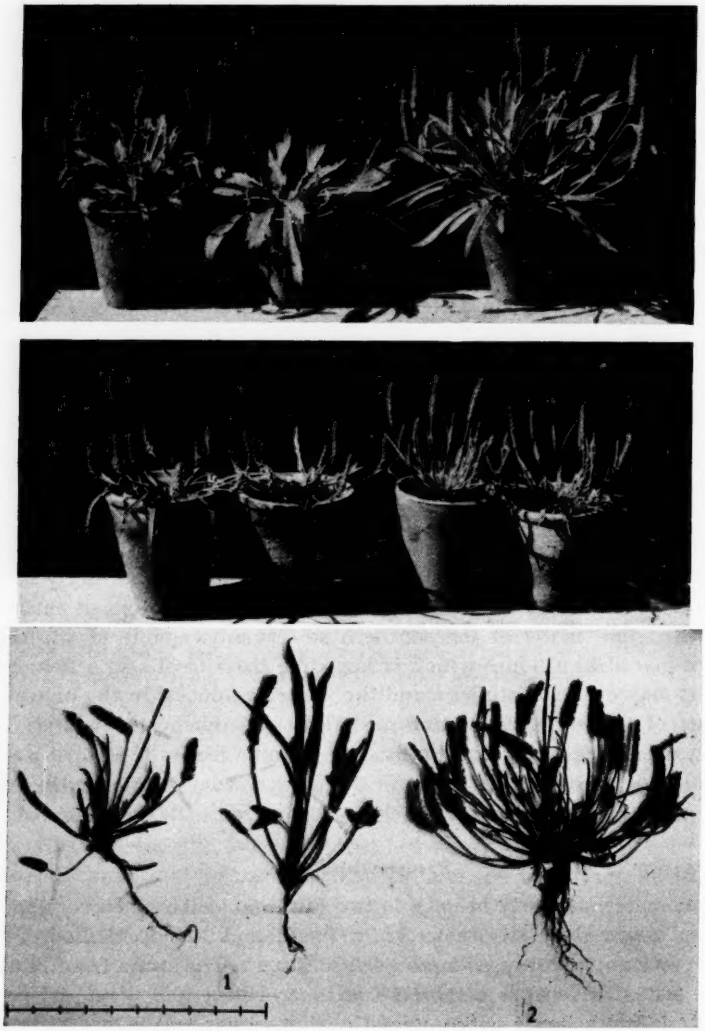

Fig. 9. Experimental cultivations 1953. Above in the middle *Plantago serraria* from Tunisia ($2n=10+3$ B); on its left *P. macrorhiza* ssp. *occidentalis* (No. 3383) from Portugal and on its right *P. macrorhiza* ssp. *macrorhiza* (No. 3289) from Tunisia. Below, the tetraploid *P. commutata*, on the left two pots and two dried specimens (1) of No. 3291 from Jerusalem, on the right two pots and one dried plant (2) of No. 3292 from Catania. The plants photographed or pressed on August 13th. Scale 10 cm.

spikes (ssp. *occidentalis* f. *hirsuta* COUT., Figs. 6 and 9). All had almost adpressed primary leaves and in the Algarve strains they were greyish green. During the summer of 1953 the weight of dry matter constantly rose; the percentage increase was 17 in the period from July 3th to August 12th and 135 from the latter date to Sept. 17th.

By the life-form and the lateral lobes ssp. *occidentalis* approaches to *P. coronopus*, but as far as our material gives at hand it is a distinct and separate unit, which deserves its rank or even ought to be regarded as a species. On the other hand, the material cultivated as No. 3073 urges to caution. The plants in question had longer leaves than are found in *P. macrorhiza* and they resembled the tall southern races of *P. coronopus* in many characters. Some of the individuals were hairy and could hardly be distinguished from some strains of *P. coronopus* (*e. g.*, No. 3671 with breadths of rhachis up to 1,7 cm.). A single plant had succulent and glabrous leaves and, apart from the size, it very much resembled the typical *P. macrorhiza*. This material seems to be the result of a cross between the two species, and studies in the herbarium confirm the view that such intermediate and probably hybridogenous types occur in many places, a fact which might even cast doubt upon the specific rank of *P. macrorhiza*. But as far as we can judge, *P. coronopus* and *P. macrorhiza* form a coenospecies and within this we are inclined to regard ssp. *occidentalis* as a third species.

According to MEROLA (1954), *P. macrorhiza* from the isle of Procida is a perennial xerophyte with summer dormancy and distinct from *P. coronopus*.

P. commutata GUSS.

This species, as mentioned above, is separated from the two others by its chromosome number and morphology. Among the morphological characters the ratio leaf length : scape length $\left(\frac{1}{2}\,B : L—S\right)$ is of some importance. In *P. coronopus* this ratio is 0,66 (Table 1) and in *P. commutata* it is 0,9 (all six experiments with the two strains Nos. 3291 and 3292 in Table 4). The ratio scape length : spike length is also low in *P. commutata* as compared with most strains of *P. coronopus*.

The two strains, Nos. 3291—3292, are strictly annuals and very early. They flower late in May, hardly two months after germination (Fig. 8). When the flowering in spring has ceased, many leaves and scapes with ripe spikes wither and are shed. Consequently the weight of dry matter

decreases much in the first part of the summer. Later, in August—September, it increases again in connection with a second flowering.

The two strains belong to two different races. No. 3291 from Jerusalem may belong to var. *rigida* PILG. It has decumbent, glabrous, succulent leaves and decumbent-ascendent scapes. The other strain from Catania (No. 3292) has very narrow, almost linear leaves, which are not succulent, but like the scapes are ascending (Fig. 9). It may be related to what has been called f. *weldenii* (RCHB.) PILG.

The Plantago serraria group

To this group belong *P. serraria*, *P. aschersonii*, *P. peloritana*, and perhaps even *P. crassifolia*, which like *P. serraria* has large chromosomes. Apart from a single culture of *P. crassifolia* we have only studied six strains of *P. serraria*. Four of these were cultivated simultaneously in Exp. 4 and are considered in Table 4. It appears that the variation is very moderate and that no correlation between the size of the plants and the number of B-chromosomes seems to exist.

SUMMARY

(1) *Plantago coronopus* and allied species have the basic number 5 and the group contains diploids, tetraploids, occasional hexaploids as well as trisomic diploids and diploids with one or three B-chromosomes (accessory chromosomes) which are heterochromatic.

(2) In the trisomic *P. coronopus* the three homologous chromosomes (β-chromosomes) may differ structurally as they in diakinsesis and at metaphase I most frequently form a bivalent and a univalent. The bivalent is lagging during anaphase I and as distinct from the other bivalents it has one localized chiasma.

(3) In *P. serraria* a tetraploid plant with two B-chromosomes was found. During meiosis its two accessories formed a pair which, however, already was separated during the early prophase. In most cells at anaphase I the two B-chromosomes were distributed to the same pole. The high frequency of multivalents indicates that the plant was an autotetraploid.

(4) It is discussed whether B-chromosomes might arise through changes of such supernumerary chromosomes as are found in *P. coronopus* and there occur as univalents.

(5) The tetraploids from the eastern part of the Mediterranean area

have a very regular meiosis and no multivalents. They are referred to *P. commutata*, which is considered an independent species.

(6) A simple doubling or even triplication of a chromosome set or the doubling of a single chromosome like the occurrence of one, two or three B-chromosomes, have only a very slight influence on the morphology of the plants and only affect quantitative characters. Chromosome races of this kind, therefore, can only be estimated as forms or even may not be distinguished as discrete taxa. On the other hand, if a tetraploid, as in *P. commutata*, has a perfectly regular meiosis without multivalent formation, this fact gives important support of a taxonomical distinction of the tetraploid as an independent species. Also the size of the chromosomes seems to be of great value from a taxonomical point of view.

(7) Through cytological studies combined with experimental cultivations it has been possible to separate two species groups, *viz.* the *Coronopus*- and the *Serraria*-group. The former comprises *P. coronopus*, *P. macrorhiza*, and its ssp. *occidentalis*, which together seem to form a coenospecies. *P. commutata* may be a member of another coenospecies in that group.

(8) The range of variation in *P. coronopus* is very wide and is best expressed through a number of clines running from the north to the south in Europe. Strains of southern origin deviate from those from the north by often being of greater size, with more ascending leaves and scapes, wider leaf-rhachis and longer spikes. They further seem to be more resistant to drought. Extreme dwarf races occur in the northwest on exposed rocks and ocean bluffs. They constitute an ecotype which differs from another similar ecotype occurring on the sea coast cliffs in South Scandinavia. The few cultivations of *P. macrorhiza* and *P. commutata* appear to reveal a similar variability in these species, whereas the range of variation in *P. serraria* perhaps is more limited.

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CHROMOSOME NUMBERS AND MEIOSIS IN CERTAIN SALICES

By *ARTUR HÅKANSSON*
INSTITUTE OF GENETICS, LUND, SWEDEN

INTRODUCTION

THE first paper with exact chromosome numbers of *Salix* species was published by BLACKBURN and HARRISON (1924). The base number 19 was found in *Salix* as well as in *Populus*. Several species investigated were diploid, but a tetraploid (*S. cinerea*) and a hexaploid (*S. phylicifolia* ssp. *nigricans*) were also found. BLACKBURN and HARRISON, however, reported a second base number in *Salix*, 22. In *S. triandra* they found two numbers, $n=19$ and $n=22$, in *S. phylicifolia* ssp. *weigeliana*, one number, $n=44$. Nordic cytologists have always found the latter species to be a hexaploid of the 19-series ($n=57$). On the other hand the number 44 was confirmed by WILKINSON (1944) who, it appears, has obtained very good root fixations. He has also reported a tetraploid form of the 22-series ($n=44$) in *S. triandra*, which is thus known to have three different chromosome numbers, and in *S. glauca*. He shares the view of BLACKBURN and HARRISON that the number 22 is derived from 19 through fragmentation of a large »compound chromosome»; he writes: »those species of the 22-base are almost certainly derived by fragmentation from the 19-paired species».

Unreduced chromosome numbers are determined in roots emerging from cuttings placed in water, reduced numbers at meiosis in ♂♂. WILKINSON (1941 and 1944) has succeeded in chromosome morphological studies. *Salix* chromosomes are small, the largest $1{,}_{75}\ \mu$, the smallest $0{,}_{50}$—$0{,}_{75}\ \mu$. WILKINSON uses seven different symbols for the various types of chromosomes in the complements, of which the main categories are L′, M, T, and S chromosomes.

WILKINSON (1944) emphasizes that »the great majority of chromosomes are alike in morphology in all species». This common type is called M chromosome: it is of medium size and has a median constriction (centromere). All investigated species also have a few L′ chromosomes, large chromosomes with a secondary constriction, satellite chromosomes (T), and S chromosomes, small chromosomes lacking

constriction (subterminal centromere). Most diploid species have 4 L′, 4 T and 2 S, the idiogram being 4 L′+28 M+4 T+2 S.

Thanks to the activity of British cytologists the chromosome numbers of all native species (20) in the new Flora of the British Isles are known. MARKLUND has determined the chromosome numbers of the majority of Scandinavian species, his numbers being published in the Scandinavian Flora of HOLMBERG and in chromosome lists of LÖVE and LÖVE. Our knowledge of Central European species is fragmentary where species lacking a wide distribution are concerned. Japanese investigators have recorded a number of *Salices* from Eastern Asia. The numbers of many *Salix* species are unknown: most of them are in subtropical regions or other regions where cytological investigations do not seem to occur. The highest degree of polyploidy that was found is octoploid (*S. glauca*, *S. borealis*) and decaploid (*S. myrsinites*).

Intraspecific polyploidy is known in *Salix*. The first case reported was *S. aurita*, which is diploid and tetraploid. Later diploid and tetraploid cytotypes have been found in *S. lapponum* and in *S. caprea*, and diploid and decaploid cytotypes in *S. myrsinites*. In *S. caprea* MARKLUND found a triploid; a similar shrub was found by WILKINSON in the usually diploid *S. daphnoides*. WILKINSON stresses that there are no taxonomical differences between the different cytotypes (see also Flora of the British Isles). Surely in *Salix* we have true intraspecific polyploidy!

My own contributions to *Salix* cytology are based on the experimental material of Professor HERIBERT NILSSON (HÅKANSSON, 1929, 1933, 1938, 1944). The good chromosome pairing at first metaphase and the regular first anaphase of simple and complex hybrids with an even number of genomes were a striking fact. This general regularity of meiosis did not, however, exclude the formation of diploid or polyploid pollen grains — in *S. viminalis*×*S. caprea* in a frequency of about 1 % of functioning ♂-gametes, to judge by the number of triploids in the progeny. In the crosses shrubs with unexpected and striking exterior, which NILSSON called »extravagant forms», had occasionally appeared. They were interpreted by him in a purely Mendelian way as extreme recombinations. However, several extravagant forms were polyploid and thus genome mutations rather than recombinations; instances are the triploid *gigantea* and *falcifolia* types, the tetraploid *neocinerea*, and the pentaploid *laurina*, all of them having appeared in the F_2 generation of *S. viminalis*×*S. caprea*. Another experimental polyploid was the extravagant form *amerinoides* from the cross *S.* (*viminalis*×*S. repens*)× *S. repens*; it was tetraploid.

Some of the results of my further investigations have been published only in papers of NILSSON, some have remained unpublished. The results in the present paper are, as a rule, obtained from new fixations made in 1952—1954; all are from shrubs growing in the Saliceta of Professor NILSSON. A key to some complex hybridizations is given on p. 481.

SOME NATURAL SALIX SPECIES

S. aegyptiaca L. — Under the name of *S. Medemii* BOISS. this species has been investigated by WILKINSON (1944). He found 76 chromosomes in roots. As to the tetraploidy I have confirmed this in ♂ meiosis, 38 chromosomes being counted in plates of first anaphase.

S. alba L. — This is a tetraploid species. WILKINSON (1941) reported four T chromosomes, two having the size of an M chromosome, two of an S chromosome. I have not investigated roots of *S. alba*, but in the roots of a hybrid *S. viminalis*×*S. alba* only two T chromosomes (satellited chromosomes) of different size were clearly seen (Fig. 1). As *S. viminalis* also has four T. chromosomes, these observations on their hybrid confirm the claims of WILKINSON (1944) with regard to the occurrence of amphiplasty in *Salix* hybrids. In a number of cases he found only two T chromosomes in the hybrid, though the parents each had four. The secondary constriction is visible in the L chromosome of Fig. 1.

S. caesia VILL. — This is a high-mountain species, in Europe occurring in the Alps above 1700 m. (HEGI). It is a dwarf willow with a slightly creeping habit. There is no doubt that this is a tetraploid species. Two shrubs of the hybrid *S. caesia*×*S. phylicifolia nigricans* were pentaploid, and roots from a *S. caesia* shrub had about 76 chromosomes. This species has not been investigated previously.

S. glabra SCOP. — A Central European species from the Eastern Alps, previously not investigated. Roots showed 38 chromosomes, meiosis 19 II (Figs. 2—4).

S. gracilistyla SER. — This is a species from Japan. Meiosis showed 19 II (Fig. 7). Previously SINOTÔ has investigated meiosis, WILKINSON roots; both report diploidy.

S. grandifolia SER. — A Central European species, not investigated previously. Meiosis showed 19 II (Fig. 8).

S. incana SCHRANK. — A species from Central and Southern Europe with, as it seems, unknown chromosome number. I have investigated a diploid hybrid *S. incana*×*S. hastata* and, thus, no doubt *S. incana* is diploid.

S. integra THUNB. — This is a Japanese species. Some hybrids showed that the species is diploid. This has previously been shown by NAKAJIMA.

S. phylicifolia s. lat. — NILSSON has crossed the nearly related *S. phylicifolia* L. and *nigricans* SM. and is of the opinion that their typical forms are only the extremes of a continuous population. Thus they cannot be considered separate species.

ssp. *nigricans* HERIB. NILSS. — Several investigators have found *S. nigricans*, investigated by certain British and Japanese authors under the name of *Andersonia*, to be hexaploid. This was also the case with two forms, collected by NILSSON in Lapland mountains; IP 40 and IP 42. Other chromosome numbers, however, are known. MARKLUND has found in Central Sweden a diploid *nigricans* form (LÖVE and LÖVE, 1948, and oral communication by Dr. MARKLUND). The ssp. *borealis*, on the other hand, he has found to be octoploid.

ssp. *weigeliana* (WIMM.) HERIB. NILSS. — Shrubs from Dovre in Norway (IO 8), from Dalecarlia (ID 55) and a form termed IH 42 were all hexaploid, roots showing $2n=114$. The derived number $n=44$, reported by BLACKBURN and HARRISON and by WILKINSON, has not been observed; also ssp. *evanescens* is hexaploid (LÖVE and LÖVE, 1948). The number 44 has been assumed to be the result of fragmentation of the L chromosomes in an ordinary tetraploid.

S. dasycladoides HERIB. NILSS. — This is not a natural species; it was artificially produced by NILSSON. It has been produced from *nigricans*×*weigeliana* after pollination from *viminalis*×*caprea* and is very similar to *S. dasyclados*. As to *S. dasycladoides*, I have investigated this species previously (HÅKANSSON, 1933); a regular meiosis was found but the chromosome number could not be determined accurately, no stages of first anaphase being obtained. Two hybrid shrubs were thought to be tetraploid but later investigations rather indicate hexaploidy. Figs. 5 and 6 show the anaphase plates from the ♂ shrub 1652. There are about 57 chromosomes in each plate. The production of hexaploid shrubs in the cross indicates the function of a triploid sperm from *viminalis*×*caprea*; unions of three tetrad nuclei have been observed (HÅKANSSON, 1929).

S. reinii FRANCH. et SAV. — This is a Japanese species with rather large leaves, not previously investigated. Plates of the first anaphase in anthers showed 76 chromosomes (Fig. 9). Thus this species is octoploid. First metaphase was so far irregular that a bivalent was often

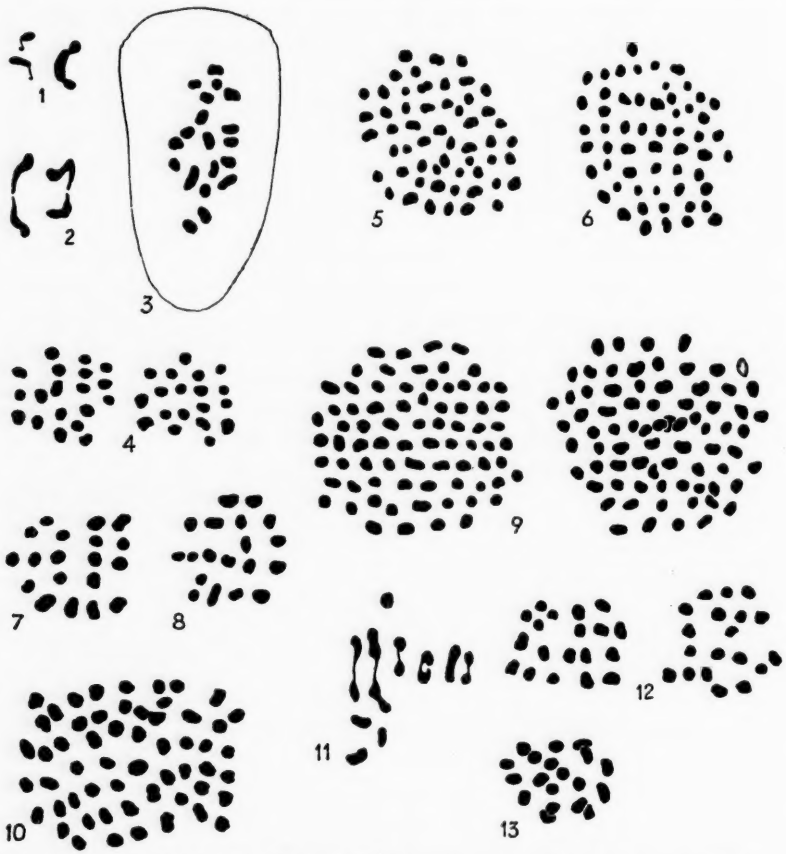


Fig. 1: *S. viminalis*×*S. alba.* The satellite chromosomes and an L chromosome. — 2: *S. glabra.* L bivalents. — 3: *S. glabra.* Prometaphase in the p. m. c. 19 II. — 4: *S. glabra.* A 1, 19+19 chromosomes. — 5 and 6: *S. dasycladoides.* The two plates of a first anaphase. — 7: *S. gracilistyla.* 19 II. — 8: *S. grandifolia.* 19 II. — 9: *S. reinii.* A 1, 76+76 chromosomes. — 10: *S. stipularis* ♀, 57 II. — 11: *S. viminalis*×*S. daphnoides.* 4 I at first metaphase. — 12: *S. viminalis*×*S. daphnoides.* A 1, 19+19 chromosomes. — 13: 5-hybrid. Prometaphase, 19 II.

outside the plate, perhaps because of lack of space (»crowding»). Lagging of a bivalent was observed at first anaphase.

S. silesiaca WILLD. — As the name indicates this is a Central European species. It seems a difficult cytological object: no roots were obtained and no usable meiotic stages. No doubt, the species is diploid, since

roots of *S. silesiaca*×*S. glabra* showed plates with 38 chromosomes. *S. silesiaca*×*S. aegyptiaca* is triploid.

S.×*stipularis* SM. — Concerning the origin of this very striking form, Flora of the British Isles remarks »variously regarded as a form of *S. atrocinerea* (*cinerea*)×*viminalis* and *dasyclados*×*viminalis*». In a Salicetum of NILSSON ♀ and ♂ shrubs were growing. Fig. 10 shows a first metaphase plate from the ♀, where 57 II are clearly visible; plates in roots confirmed the hexaploidy of this shrub. The ♂ shrubs were no doubt also hexaploid; this was evident from first anaphase plates showing 57 chromosomes. One or a few univalents may occasionally occur.

S.×*stipularis* may have arisen through the doubling of the chromosomes of a triploid hybrid. *S. dasyclados* exists in a tetraploid and a hexaploid cytotype (MARKLUND, cited after LÖVE and LÖVE, 1942) and thus the hexaploidy of *stipularis* does not decide between the two alternatives of its origin mentioned above.

Conclusions. — Through the determination of the chromosome numbers of *S. caesia*, *S. glabra*, *S. grandifolia*, *S. incana*, and *S. silesiaca* the chromosomes of the Central European *Salix* flora are somewhat better known, only a few species being now uninvestigated. A large preponderance of diploid species is evident. A morphological analysis of *Salix* idiograms was not attempted, although chromosome types discovered by WILKINSON have been recognized. Size differences may also be evident in meiosis, where two particularly small bivalents, and one or two large ones may be discerned. No evidence of so-called diffuse centromere was obtained. M chromosomes are clearly monocentric; although I have not been able to discern the centromeric constriction, I have observed at anaphase the centre turned towards the pole, the ends towards the equator. This definitely speaks against a diffuse or polycentric centromere. The postulated changes in chromosome number through fragmentation are improbable in monocentric chromosomes. No instance of a 22-series has been met with and all investigated *phylicifolia* had the haploid number 57.

MEIOSIS IN SOME DIPLOID SALIX HYBRIDS

S. depressa×*S. caprea.* — A ♂ shrub was investigated. Meiosis was very regular. First metaphase showed 19 II, first anaphase showed no lagging.

S. aurita×*S. caprea.* — A ♀ shrub was investigated. First metaphase

showed 19 II but occasionally 2 I were seen, indicating a very weak asyndesis.

S. incana×*S. hastata.* — Two ♀♀ were investigated. Also here 19 II or occasionally 18 II+2 I were observed.

S. viminalis×*S. daphnoides.* — An earlier investigation had shown a weak asyndesis in this hybrid (HÅKANSSON, 1938). Further investigation has confirmed this. The most common pairing was 19 II, but 18 II+2 I, or more rarely 17 II+4 I, also occurred. In two anther loculi 4 I were counted in 2 p. m. c. (Fig. 11), 2 I in 10, while 35 p. m. c. had 19 II. First anaphase often showed some stickiness.

A 5-hybrid. — This hybrid had been produced through crossing a spontaneous hybrid *cinerea*×*purpurea* with *S. aurita* and then this tertiary hybrid with *viminalis*×*caprea* (NILSSON, 1930). Anthers of a ♂ shrub I fixed in 1934. Meiosis showed 19 II, though rather frequently the pairing was 18 II+2 I (Fig. 13).

NILSSON remarks (1930, p. 5) that the original *cinerea*×*purpurea* was not quite typical. Clearly the rather numerous I's presumably present in this original hybrid had been lost after two consecutive crosses with diploids.

Conclusion. — The chromosomes of eight different diploid *Salix* species have now been shown to effect pairing in different hybrid combinations. These species are *purpurea*, *hastata*, *aurita*, *caprea*, *viminalis*, *depressa*, *repens*, *incana*, and *daphnoides*. In certain cases a weak asyndesis was observed. This is perhaps sometimes due only to low temperature, in other cases it is probably constitutional as in *viminalis*×*daphnoides* and in the 5-hybrid. The homology of different *Salix* genomes seems, however, clear.

MEIOSIS OF SALIX VIMINALIS×SALIX ALBA

Hybrids between species belonging to different sections are difficult to produce and very rare in nature. I have previously investigated *S. triandra*×*S. viminalis* (HÅKANSSON, 1944) and have now been able to investigate a second such hybrid, produced by Professor NILSSON. *S. viminalis*×*S. alba* has long grown in his *Salix* cultures though it is now dead. The shrub was triploid, roots showed 57 chromosomes, a number to be expected (*cf.* p. 456). It has rarely flowered but in 1950 numerous catkins were produced and embryological-cytological investigations were possible.

The ovules are sterile. In many cases meiosis does not occur but more

often four macrospores are formed, though as a rule all degenerate. Only rarely does the chalazal macrospore show a tendency to form an embryo-sac. The cell shown in Fig. 16 has a very large nucleus with two nucleoli. It may be that this nucleus is unreduced, since the remains of only one further spore were seen. As meiosis shows many I's, such observations indicate the forming of a restitution nucleus in this ovule. There were no organized embryo-sacs in the ovules when the stigmata were conceptible; no fertilizations were possible.

Meiosis is rather late, occurring after the integument has formed the micropyle. Diakinesis stages show many univalents. At first metaphase the plate is formed of trivalents, bivalents and univalents but a number of univalents are outside the plate (Fig. 14). Trivalents are relatively rare but difficult to distinguish from bivalents. Polar views indicate also a high number of bivalents, though very few clear plates were observed, owing to the small diameter of the embryo-sac mother cell. Fig. 15 shows, however, a very clear case. There are 8 I outside the plate which contains 28 chromosomes, paired and unpaired. This shows the pairing 2 III+17 II+17 I. The number of trivalents was probably somewhat larger in certain cases but is no doubt generally low. Notwithstanding the large number of univalents the first anaphase does not lead to elimination of chromosomes in a higher degree or more frequently. A few interkinesis stages observed showed no elimination.

Conclusion. — A previously investigated diploid section hybrid *S. viminalis*×*S. triandra* showed weakened pairing with 10—20 I. The triploid *S. viminalis*×*S. alba* probably shows autosyndesis between *alba* genomes; and thus a lower affinity between genomes from different species sections must find less clear expression than in the diploid hybrid. However, a comparison of the pairing in triploids from the *viminalis*×*caprea* crosses shows a considerably higher number of univalents in *viminalis*×*alba.*

SOME SALIX PHYLICIFOLIA ssp. WEIGELIANA HYBRIDS

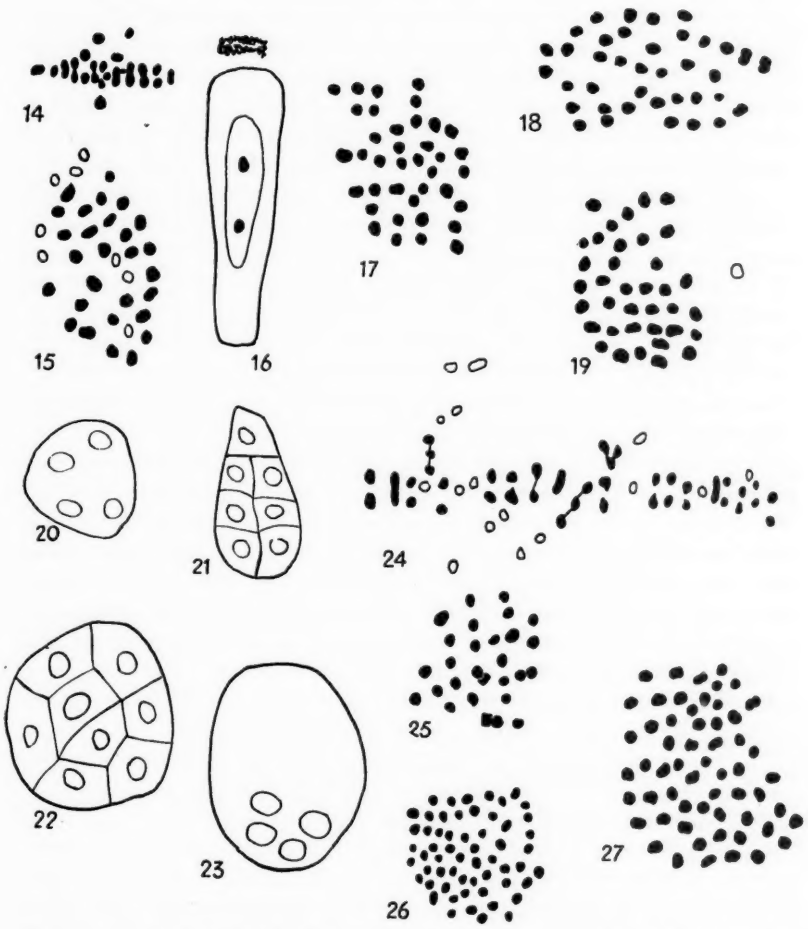
In 1938 I described meiosis in an artificial hybrid *S. viminalis*× *S. phyl. weigeliana.* This tetraploid hybrid showed a very regular meiosis, the chromosomes forming 38 II. The pairing was of the alloautoploid type, 19 *viminalis* chromosomes forming bivalents with 19 *weigeliana* chromosomes while 19 II are formed through autosyndesis of *weigeliana* chromosomes. A similarity of the genomes of *weigeliana* is indicated.

S. repens×*S. weigeliana.* — A ♀ shrub was investigated. Meiosis occurs as in most *Salix* species rather early before the formation of a micropylar canal, when the integument has hardly grown higher than the nucellus. First metaphase showed 38 II (Fig. 17). Occasionally the pairing is 37 II+2 I. Thus, autoalloploid pairing occurs also in this hybrid. First and second anaphase showed no lagging.

In the ovules a normal embryo-sac is formed. The hybrid nature of the shrub may be manifested in occasionally slight irregularities as, for instance, an excentric position of the e. m. c. in the nucellus or unusual size relations of the macrospores, but in nearly all ovules an organized embryo-sac is developed. Open-pollinated ovaries contained a number of fertilized ovules. The first stages of seed development were observed. Embryo and endosperm development was then vigorous. A two-celled embryo was observed in embryo-sacs with four endosperm nuclei, but an embryo-sac with eight endosperm nuclei already contained an embryo with 12 cells, a still larger embryo accompanied eight dividing endosperm nuclei. It has been shown in several *Salix* species that endosperm development starts before the division of the eggcell, although rather soon the embryo shows the more rapid development (HÅKANSSON, 1954). In hybrids endosperm development may be weak, the turning point occurring more early than in pure *Salix* species. In one case the ovule had not been reversed: it was orthotropous with the micropyle turned away from the placenta. This had not prevented its fertilization. The ovule contained endosperm nuclei, and traces of the pollen tube showed that it had grown on the surface of the ovule to the micropyle.

The hybrid *S. repens*×*S. phyl. weigeliana* is thus very fertile. *S. viminalis*×*S. weigeliana* had good pollen and was also fertile (NILSSON, 1930, p. 37 »wohl als gut fertil»). NILSSON (1930, p. 61) crossed these two *weigeliana* hybrids and obtained many germinable seeds and a large progeny. The latter has not been investigated cytologically; it was no doubt tetraploid, both parents forming gametes with 38 chromosomes.

S. septemgena. — NILSSON (1930) has given this name to a very complex hybrid procured from successive crosses. Spontaneous (*cinerea*×*purpurea*) had been crossed with *S. aurita* and the hybrid obtained with *viminalis*×*caprea* (compare p. 460). A 6-hybrid was the result of a cross with *S. daphnoides*, while a last cross 6-hybrid×(*viminalis*×*phyl. weigeliana*) resulted in *S. septemgena.* The latter consisted of 49 shrubs, quite sterile as was stressed by NILSSON. The hybrid *viminalis*×*weigeliana* forms gametes with 38 chromosomes. The 5-hybrid was di-



Figs. 14—16: *S. viminalis*×*S. alba.* — 14: M 1, side view. — 15: M 1, polar view, the pairing apparently was 2 III+17 II+17 I, with 8 I outside the plate. — 16: Macrospore, the nucleus was probably unreduced. — 17: *S. repens*×*S. phyl. weigeliana*, ♀-shrub, 38 II. — Figs. 18—23: *S. purpurea*×*S. phyl. weigeliana.* — 18: ♀-shrub, 38 II. — 19: aneuploid ♂, 38 II+I. — 20—23: sterile ♂-shrub, groups of archespore cells in older anthers. — 24: *S. repens*×*S. cinerea.* 3 III+16 II+16 I. — 25: *S. repens*×*S. cinerea.* M 2, 28 chromosomes. — 26: *S. cinerea*×*S. reinii* ♀ shrub, early A 1, 57 chromosomes. — 27: *S. cinerea*×*S. glabra* ♂ shrub, A 1, 57 II.

ploid (see p. 460); the chromosome number of the 6-hybrid is unknown (only one shrub appeared) but it was probably also diploid, *S. daphnoides* being a diploid species. The *septemgena* producing cross was thus diploid ♀×tetraploid ♂, *S. septemgena* consequently being a hybrid population of sterile triploid ♀♀ and ♂♂. The probability of triploidy was pointed out in an earlier publication (HÅKANSSON, 1938).

I have not studied *S. septemgena* myself but NILSSON (1930, 1953) has investigated meiosis in some ♂♂ using the aceto-carmine method. He reports the chromosome number $n=22$ and claims the production of a morphologically new *Salix* with a new chromosome number. Its sterility is, according to NILSSON, a demonstration of the difficulties encountered in forming a new species. »Eine neue Art, die morphologisch und zytologisch eine natürliche linnéische Art (Linneon nach LOTSY) täuschend nachahmt scheint also in dem *septemgena*-Typus vorzuliegen» but »die Extravaganz der *septemgena* ist indessen mit dem Preis der Sterilität erkauft und dies stürzt alles um» and »die Art ist eine ephemere, zum Verschwinden in einer Generation verurteilt» (NILSSON, 1930; *cf.* also 1953, p. 390). The production of a population containing male as well as female shrubs of a new, uniform, morphological type is no doubt of great interest but the new chromosome number cannot be correct. This should entail one parent of the *septemgena* population having only 6 chromosomes. The error of NILSSON is explainable. The triploid *S. viminalis*×*S. caprea* forms plates at first metaphase which seem to contain 21—23 chromosomes, but many of these are trivalents. The *septemgena* plates figured by NILSSON seem very similar. A tetraploid hybrid combination had probably shown better fertility, the triploidy probably underlining an inherent tendency to sterility.

S. purpurea×*S. phyl. weigeliana.* — Five shrubs, two ♀♀ and three ♂♂ of the same cross, were investigated. It is a new instance of a tetraploid *weigeliana* hybrid. ♀♀ formed as *repens*×*phyl. weigeliana* 38 II and the regularity of meiosis was striking. Here also one or two univalents were occasionally seen. The univalents may be due to the different possibilities of pairing between four structurally similar genomes (choice of partner; DARLINGTON). One ♂ shrub was hypertetraploid, first metaphase showing 38 II and a univalent (Fig. 19). The supernumerary chromosome may also form a trivalent. At first anaphase the univalent seems in most cases to pass undivided to the pole. The second division was regular except for occasional lagging.

Two ♂ shrubs only rarely showed meiosis due to a disturbance of the development in the anther loculi. This disturbance usually sets in

before meiosis when the anthers are filled with an archespore. The archespore becomes divided into cell groups of very different size and form: some are rather large, others small and often spherical, consisting of a few cells. The nuclei do not start the meiotic prophase. Cell walls often disappear and multinuclear, often spherical formations are seen; these latter increase in size (Figs. 20—23). Often larger syncytia are formed. In old anthers large bladders with a varying number of nuclei are frequent. Bladders are sometimes hypertrophied tapetal cells. Few loculi with meiotic stages were found. Meiosis was regular here and 38 II are also formed in sterile shrubs. Pollen production is poor.

Conclusion. — Three different tetraploid *weigeliana* hybrids have now been investigated. All show a regular meiosis with 38 II, occasionally one or two I's are observed. The chromosome pairing is of the alloautosyndetical kind, two *weigeliana* genomes pairing in the hybrid.

The *weigeliana* hybrids investigated here are artificial. BLACKBURN and HARRISON have investigated spontaneous *S. aurita*×*S. phyl. weigeliana.* They describe an irregular meiosis. Here the chromosome numbers of the putative parents were 38 and 44, and this hybrid is not comparable with a hybrid between diploid *aurita* and hexaploid *weigeliana.* In the latter, 38 II are expected, *S. aurita* forming 19 II with *S. caprea*, *S. caprea* 19 II with *S. viminalis*, and *viminalis* 38 II with *weigeliana.*

The male sterility observed in *purpurea*×*weigeliana* seems to be of genic nature, sterile and fertile shrubs appearing in the same progeny.

SOME SALIX CINEREA HYBRIDS

In a paper of the year 1938 I discussed the sterility of some artificial ♀ *S. cinerea* hybrids, the pentaploid (*cinerea*×*weigeliana*) and five triploids, *cinerea*×*caprea*, *cinerea*×*viminalis*, *cinerea*×(*caprea*×*viminalis*), *cinerea*×*purpurea*, and *cinerea*×*repens*. Only ♀♀ were investigated, ♂♂ only rarely appearing in *cinerea* crosses. A peculiarity in all hybrids was the very pronounced retardation of meiosis. In many ovules the embryo-sac mother cell degenerates, in others it increases in size, the large nucleus undergoing meiosis very late. Thus only very exceptionally has the ovule an embryo-sac when the stigmata are conceptible. At meiosis the number of univalents was unexpectedly high in triploid *cinerea* hybrids. It may be that the retardation and prolongation of the first metaphase make the chromosome unions looser.

S. repens×*S. cinerea.* — New material of this hybrid was invest-

igated; it was from a new cross. Meiosis has previously not been observed in this hybrid. It was found in some very large e. m. c. in ovaries with fully expanded stigmata. At first metaphase the expected high number of univalents occur. Fig. 24 shows a first metaphase with rather scattered chromosomes. There seemed to be 3 III only, thus showing the weak pairing; the number of I's was 16. No attempt was made to study chromosome pairing here more closely. Interkinesis stages observed showed no elimination. Fig. 25 shows a plate of the second metaphase with 28 chromosomes.

Investigation of young capsules showed abortion of all ovules.

S. cinerea×*S. reinii.* — This is a hybrid between a tetraploid and an octoploid species, its hexaploid chromosome number being verified in roots. Here the development in the ovules was different from other investigated *cinerea* hybrids in that it was quite normal. Meiosis was not retarded here, occurring when the growing integument had only attained the length of the nucellus. At first metaphase a regular plate is formed, consisting of bivalents. Only one or two univalents occasionally occurred. The e. m. c. is rather slender here, resulting in a rather close packing of the bivalents. Their number is doubtless often 57 though exact countings are difficult (Fig. 26). First anaphase may be regular but cases of lagging have been observed.

In the large majority of ovules four macrospores are formed, the chalazal spore growing to a normal embryo-sac. In open-pollinated capsules several ovules showed a vigorous embryo and endosperm development. Many ovules were, however, unfertilized.

S. cinerea×*S. glabra.* — This is a hybridization between a tetraploid and a diploid species. It had resulted in about forty hybrid shrubs, three of them ♂♂. A remarkable feature was that the rather complete sterility of previously investigated triploid *cinerea* hybrids was here changed to a relative fertility; the pollen seemed good in the ♂♂. In ♀ shrubs embryo-sac development showed no pronounced retardation and proceeded normally. Ovaries with conceptive stigmata had organized embryo-sacs in the ovules. In a number of ovules development was, however, slow or disturbed. After open-pollination a young embryo and endosperm were formed in many ovules, although the endosperm was often very weak.

Meiosis in two investigated ♀♀ was regular. Univalents occur here occasionally and in observed cases the first anaphase showed no lagging. Unfortunately the number of chromosomes in the plates could not be determined, but clearly the shrubs have more than 57 chromo-

somes. Roots of one ♀ indicated 114 chromosomes; this shrub was thus hexaploid. Roots of other ♀♀ allowed only uncertain counts. Meiosis of the ♂ shrub 4191 could be studied better. Here the chromosomes could be counted in the anaphase plates, which usually separated without lagging. Counts showed 57 chromosomes (Fig. 27). At first metaphase occasional univalents may be observed.

Conclusions. — Further investigations have shown the existence of two kinds of artificial *cinerea* hybrids: very sterile ♀♀ with retarded meiosis and more fertile ♀♀ showing little or no retardation. The former have an odd number of genomes, the latter an even number. It is difficult to see how this could explain the difference.

The fertile hybrid *cinerea*×*reinii* is the result of a normal fertilization and is a hexaploid with two *cinerea* and four *reinii* genomes. The pairing here may be purely autosyndetical with 19 II formed by *cinerea*, 38 II formed by *reinii* chromosomes. Probably also some allosyndetic pairing occurs. The fertile hybrid *cinerea*×*glabra* cannot be the result of a union of reduced gametes. It also seems hexaploid, which was quite unexpected, and must result from the doubling of the chromosomes, probably at the division of the zygote. This doubling cannot have occurred in the soma of the ♀ parent, because in such a case the hybrid should be pentaploid, nor is the production of unreduced gametes expected in pure species.

SALIX LAURINA AND DERIVATIVES

S. laurina (Sm.) Willd. — *S. laurina* has been monographically treated by Nilsson (1928). It exists only as sterile ♀ shrubs and its history is traced back to the end of the 18th century when it appeared in an English Salicetum. Later, through cuttings, it has obtained a wide distribution. Its hybrid nature was early recognized but concerning the putative parents very different opinions have been expressed; the majority considered it a *S. cinerea*×*S. phylicifolia weigeliana.* Nilsson had, however, procured a similar *laurina* shrub in the F_2 of his first *viminalis*×*caprea* cross (Nilsson, 1918, 1928), and claims to have shown the origin of *S. laurina.* Embryological and cytological investigations show similarity between the *laurina* »*arte facta*» of Nilsson and ordinary *laurina* »*hortensis*» but an exact determination of the chromosome number seemed almost unattainable since no good root plates were then found (Håkansson, 1929). Later, I have determined the chromosome number

of roots to about 95 (Fig. 28). *S. laurina* is pentaploid (see also NILSSON, 1935).

The embryological investigation showed a retardation of meiosis very similar to that later observed in different *cinerea* hybrids. In the large e. m. c. irregular meiosis with univalents and lagging at first anaphase seemed very clear but the number of chromosomes was obviously underestimated: it was thought to be 84—86. In a few ovules developing embryo-sacs were observed indicating the possibility of occasional fertilizations. After extensive pollinations NILSSON has subsequently shown that *laurina* is not completely sterile; he got most seeds after pollination with *phylicifolia weigeliana.*

S. laurina×*S. phylicifolia weigeliana.* — The pollinations resulted in 29 hybrid shrubs, 28 of them were intermediary between the parents and one was exceptional (NILSSON, 1935). One of the intermediaries was a ♂. I made slides from this shrub in 1934 and in 1954.

Unfortunately, as no roots appeared on the cuttings, the exact chromosome number could not be determined. At first metaphase meiosis showed an equatorial plate but also some univalents outside the plate (Fig. 29). The number of such univalents varied; six was the most common number. At first anaphase chromosome lagging was the rule and this sometimes led to elimination. Lagging univalents are usually divided and cause further lagging at second anaphase. Univalents outside the plate pass undivided to the pole (Fig. 30). Meiosis was not delayed.

Thus, meiosis is similar to meiosis in the mother plant, *laurina,* though the number of univalents seems to be smaller. The chromosome number may be the same: polar view of the first anaphase stage shows about forty chromosomes in the separating plates, together with univalents outside the plate and of laggers an approximately pentaploid number are indicated. The hybrid has arisen from a cross pentaploid×hexaploid but in *laurina* meiosis there are usually more univalents on the micropylar-directed half of the spindle. As the embryo-sac is formed of the chalazal macrospore, most and sometimes perhaps all univalents may be absent from the embryo-sac. No retardation of meiosis was observed in this ♂ *laurina.* It is more fertile than *S. laurina* having about 50 % good pollen (NILSSON, 1935).

S. superlaurina. — This was the exceptional shrub in the progeny of *S. laurina* which resulted from a cross with *phylicifolia weigeliana.* It was a ♀ shrub with exceptionally large catkins and rather large leaves. It was fertile, the capsule becoming filled with seeds (NILSSON, 1935).

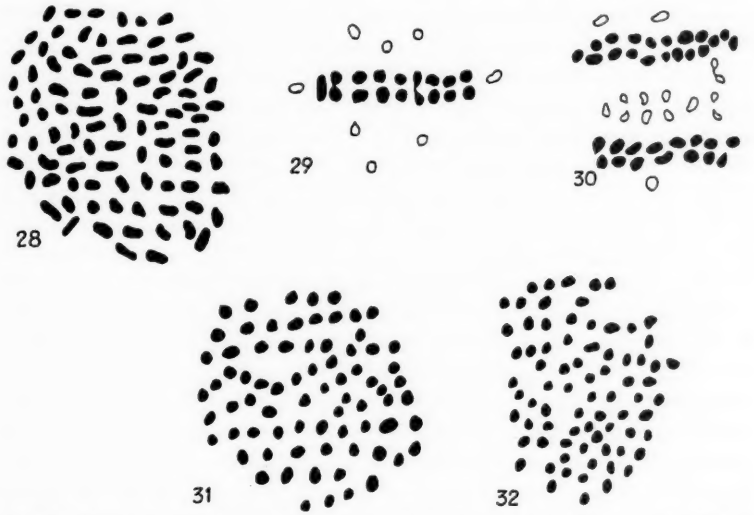


Fig. 28: *S. laurina* »*arte facta*». Root plate, 95 chromosomes (about). — 29: *S. laurina*×*S. phyl. weigeliana* ♂ shrub. M 1, univalents in and outside the plate. — 30: A 1, a number of univalents divide. — 31: *S. superlaurina.* ♀ A 1 plate, 76 chromosomes. — 32: *S. superlaurina.* M 2, 76 chromosomes.

Investigation of root tips indicated octoploidy, about 140 chromosomes being counted.

Investigation of ovule development has shown that an embryo-sac is formed in most ovules. In a number of ovules the e. m. c. degenerates but meiosis more usually sets in. The retardation observed in *laurina* does not occur here. A macrospore tetrad of the usual T-type is formed and the chalazal macrospore grows to an organized embryo-sac, which was often fertilized in slides of a larger ovary with withered stigmata. Ovules and embryo-sac are rather large.

At first metaphase a regular plate is formed, consisting apparently of bivalents. Occasionally a univalent is seen, and probably some multivalent formation may occur. Owing to the size of the e. m. c. some very clear plates were found. Fig. 31 shows a first anaphase plate with 76 chromosomes, the same number being found in the second plate, not figured here. Fig. 32 shows a second metaphase from the upper dyad cell. Also here 76 chromosomes are evident.

Thus the octoploidy of *S. superlaurina* cannot be doubted. It is from the same pentaploid×hexaploid cross as ♂-*laurina* but here an un-

reduced eggcell has been fertilized. The production of a pentaploid embryo-sac in *S. laurina* may have been effected by means of a restitution nucleus. The considerable lagging observed at first anaphase may, in rare cases, have such a result. On the other hand the chromosome doubling may have occurred at second anaphase. In *laurina* as well as in other *Salices* with retarded meiosis the spindle is often transverse in the chalazal-directed dyad cell. This very unusual orientation of the spindle is due to the large diameter of this cell in such cases of delay (HÅKANSSON, 1938). But the space is no doubt restricted, and this may facilitate the forming of a restitution nucleus at the second division.

S. dasylaurina. — This new form was produced by NILSSON from *S. superlaurina* through a cross with *S. dasycladoides*, the quadruple hybrid *S.* (*phylicifolia weigeliana*×*phylicifolia nigricans*)×*S.* (*viminalis*×*caprea*). The resulting hybrids were very vital and fertile, and showed rapid growth; they were on the whole very robust (NILSSON, 1953, p. 408). A cross of two shrubs has resulted in a large *dasylaurina* population, and, to use his own words, NILSSON has »eine hochgradig fertile und kräftige neue Art experimentell hergestellt». NILSSON finds it very probable that *dasylaurina* has sometimes, perhaps often, arisen in nature though it has never been described by any taxonomist nor collected in a herbarium. Because it does not seem to exist in nature, »so kann ihre Abwesenheit da nicht andres erklärt werden, als dass sie dort nicht existenzfähig ist». NILSSON finds here a new instance to support his thesis of the impossibility of the origin of new species in nature.

The cytological investigation of *dasylaurina* is somewhat preliminary. Meiosis has been studied in the male shrubs X, XIII and XV, all from the first generation. As the data have been collected this year, their publication must be reserved for a future paper. It may be mentioned, however, that at first metaphase there was a variable, usually small, number of univalents. First anaphase showed lagging univalents as well as univalents early approaching the pole. The anaphase plates had sixty or more chromosomes and the observations indicate that the shrubs are heptaploid. Roots of the shrub XV had plates with about 133 chromosomes. As *dasycladoides* seems hexaploid (compare p. 457) this is expected, the cross being octoploid ♀×hexaploid ♂.

Conclusions. — Two very interesting vital and fertile new *Salices* are derivatives of the nearly completely sterile *S. laurina.* One of them, *S. superlaurina*, has the nature of a genome mutation, its origin also proving the existence of functioning ♀ gametes with doubled chromo-

somes. The other, *S. dasylaurina*, arose as a hybrid population, containing male and female shrubs.

NILSSON (1935) supposes the mechanism leading to the origin of *S. laurina* to be the production of tetraploid ♂ gametes in *S. viminalis*×*S. caprea* through the union of all tetrad nuclei. This seems plausible. As NILSSON pointed out, in such a case *laurina* should contain two complete *viminalis* genomes, two *caprea* genomes and a mixed genome with *caprea* and *viminalis* chromosomes. Thus, *S. laurina* is a doubled F_1 with a fifth mixed genome. The pentaploidy is also consistent with older theories of *laurina* being a *cinerea* hybrid under the assumption that the other parent species is a hexaploid. The cytology of the pentaploid *cinerea*×*phylicifolia weigeliana* is very similar to meiosis in *laurina*. Remarkable is the retardation of meiosis occurring in the two compared forms (see HÅKANSSON, 1938). Such a retardation was not observed in triploid *viminalis*×*caprea* and thus one would hardly expect it in the pentaploid. The evidence against the *cinerea*×*phylicifolia* theory is the experimental production of *laurina* from *viminalis*×*caprea*. NILSSON has also stressed that the hybrid *cinerea*×*weigeliana* is not similar to *laurina* (1928, p. 46).

S. superlaurina has five genomes from *laurina* and three *weigeliana* genomes. Regular meiosis is expected here. The pairing observed may be 19 *caprea* II, 19 *viminalis* II, 19 *weigeliana* II and 19 II, formed of *weigeliana* chromosomes pairing with *viminalis* and *caprea* chromosomes. But also the fertilization of an unreduced eggcell of a *cinerea*×*weigeliana* hybrid with a *weigeliana* gamete ought to result in a product with normal pairing, 19 *cinerea* II, 57 *weigeliana* II. The introduction of three *weigeliana* genomes has in either case resulted in a cancellation of the meiotic retardation of *laurina*.

S. dasylaurina has no new chromosomes; the univalents at meiosis are the result of the uneven number of genomes. An investigated ♀ shrub did not show meiotic retardation.

SOME HIGHLY COMPLEX HYBRIDS

Here some hybrids with increasing complexity are treated. They are the results of successive crossings, the last being a 14-hybrid (or 13-hybrid, *weigeliana* and *nigricans* being subspecies).

S. polygena. — The genesis of this hybrid has been described by NILSSON (1935; 1953, p. 395). It is an 8-hybrid, formed from two quadruple hybrids, *S.* (*purpurea*×*daphnoides*)×(*aurita*×*repens*) and

S. (*phylicifolia nigricans* × *phylicifolia weigeliana*) × (*viminalis* × *caprea*). The former hybrid is diploid and in meiosis shows 19 II (HÅKANSSON, 1938). The latter hybrid unfortunately proved a difficult cytological object. The chromosome number could not be determined exactly, but it was thought that *S. dasycladoides*, as the hybrid has been called, was tetraploid (HÅKANSSON, 1933). Further investigations show that this number was too low, probably all shrubs being hexaploid (see p. 457).

The reciprocal crosses producing *polygena* resulted in a low number of good seeds: lethality among seedlings further reduced the progeny and only six shrubs attained larger size. One ♂ shrub was tetraploid and had regular meiosis whereas the chromosome number of a ♀ shrub B was more uncertain (HÅKANSSON, 1938).

Four of the original shrubs have now been investigated. *Polygena* has different chromosome numbers. Tetraploidy was confirmed in the previously investigated ♂ shrub A. On the other hand the ♂ E proved to be hexaploid. It is a much more robust type, now growing in the form of a small tree. It still produces catkins profusely. Meiosis is very regular, first metaphase showing perfect pairing. No lagging occurred at first anaphase, the plates showing 57 chromosomes (Figs. 33 and 34). The ♀ shrub D is now in a poor condition. It is, as in the case of E, a small tree but only some roots could be investigated. They indicated a pentaploid or rather hexaploid chromosome number.

A and B are from the cross diploid ♀ × hexaploid ♂; here only reduced gametes (A) or possibly an unreduced eggcell (B) had functioned. D and E are from the reciprocal cross hexaploid ♀ × diploid ♂; here triploid male gametes have been formed and performed fertilizations (compare the origin of *S. dasycladoides*).

S. polygena is fertile and the crossing of two shrubs has resulted in a large progeny. NILSSON has enumerated five different points which make the *polygena* population »equivalent to a natural allogamous species». The fifth point, however, concerning the chromosome number is now invalid. NILSSON stresses that *polygena* shows all the characteristics of a Linnean species. He has, however, also demonstrated the poor vitality of *S. polygena*, which was still more pronounced in its progeny, »a *polygena* population would never have been able to survive as part of the wild-growing flora, thus it is unable to contribute to a further evolution» (NILSSON, 1953, p. 1185).

The 10-hybrid. — This hybrid has arisen through the cross *S. polygena* D ♀ × *S.* (*silesiaca* ♀ × *aegyptiaca* ♂) ♂. I have recently investigated the male parent but the results are unpublished. Three *silesiaca* ×

aegyptiaca shrubs were, as expected, triploid. Meiosis showed the occasional production of triploid pollen grains. The cross had only resulted in two 10-hybrids, one a large and vigorous ♂ shrub, the other small (oral communication of Professor NILSSON). The former is now the only shrub surviving. Such a high hybrid does not probably have chromosomes from all species used at its production. In the meiosis of a species hybrid with allosyndesis many chromosomes are lost and in the successive crossings all the chromosomes of certain species may have disappeared. On the other hand segments of lost chromosomes may be preserved, transfered through crossing-over to homologues of other species still present in the hybrid. By the production of gametes with a doubled chromosome number all chromosomes may be preserved, namely, in cases of doubling through a restitution nucleus or at the second division, if nuclei of different spindles are united and the effect of crossing-over is ignored. Unions of the nuclei of only one second division spindle do not restore loss at the first division.

The 10-hybrid was hexaploid. After much work good plates of first metaphase and anaphase were procured (Fig. 35), 57 chromosomes were counted in such plates. The pollen was good, being formed of grains of equal size.

The 10-hybrid is the result of the fertilization of a triploid eggcell from *polygena* D and a triploid sperm from *silesiaca*×*aegyptiaca*.

The 12-hybrid. — This hybrid has been produced through the pollination of *S. grandifolia*×*S. gracilistyla* with pollen from the 10-hybrid. As has been shown above, the two new species brought into the cross are diploids. This is a diploid ♀×hexaploid ♂ cross. It has resulted in a large progeny, some 80 shrubs growing in the experimental field. They are unfortunately all ♀♀ — unfortunately, because meiosis is more difficult to investigate in ♀-shrubs.

Meiosis was studied in three shrubs, 4732, 4798 and 4805. The latter was a larger and seemingly more vital shrub than the rest. However, the three shrubs had the same chromosome number, being tetraploid. Meiosis was regular. First metaphase showed a regular equatorial plate; only occasionally were univalents observed. They were due to multivalent formation. Side views showed an occasional trivalent or quadrivalent and their presence makes chromosome counts in the first metaphase plates difficult. The plate shown in Fig. 36 has, as it seems, 36 II+IV; Fig. 37 has 38 II. The first anaphase is regular; lagging was not observed, but once a bridge united the telophase groups. The second division was also regular.

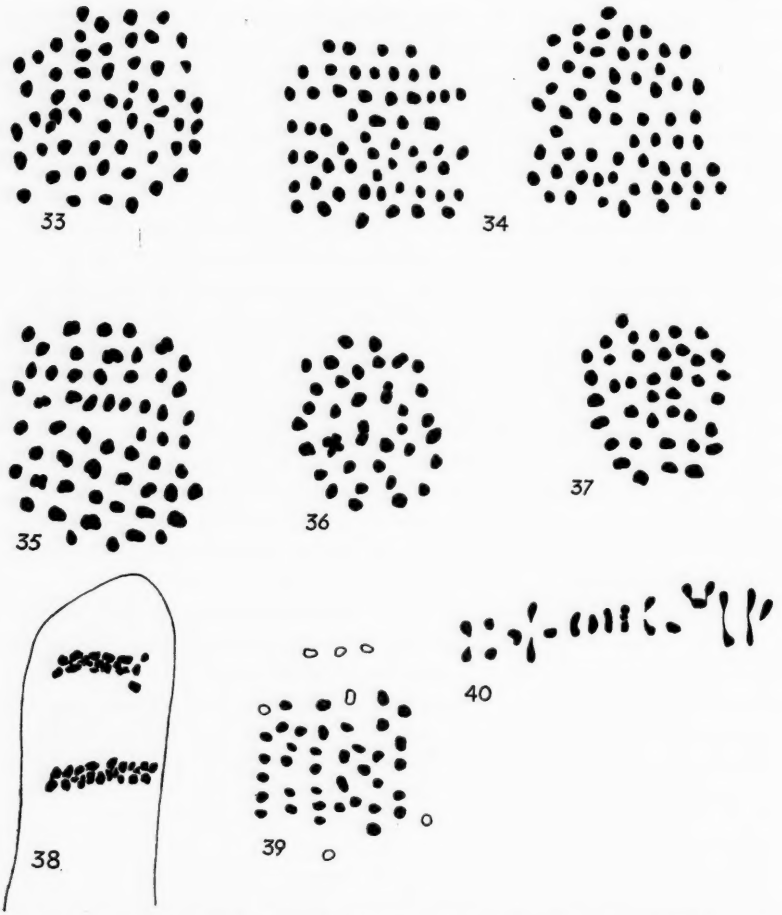


Fig. 33: *S. polygena* E, M 1, about 57 II. — 34: *S. polygena* E, A 1, 56+57 chromosomes. — 35: 10-hybrid, 57 II. — 36: 12-hybrid, IV+36 II. — 37: 12-hybrid, 38 II. — 38: 12-hybrid. A 1. — 39: 14-hybrid. M 1, 8 I outside the plate. — 40: 14-hybrid. M 1, all chromosomes were not figured, III, II and I occur.

The development of the embryo-sac proceeds normally in the 12-hybrid. In a minority of the ovules the e. m. c. never divides but in most cases meiosis occurs with no greater delay. Many ovules contain at the time of pollination an organized embryo-sac of normal appearance.

Young capsules of 4732 and 4805 were investigated; in the ovules in

most cases a vigorous development of embryo and endosperm was observed (HÅKANSSON, 1954).

The 14-hybrid. — This is the result of a cross 12-hybrid ♀×(*S. cinerea*×*glabra*) ♂. The cross resulted in 10 shrubs, all ♀♀. *S. cinerea*× *S. glabra* is, as mentioned on p. 466, (at least partly) hexaploid, and this was a tetraploid ♀×hexaploid ♂ cross in all probability.

Five shrubs were investigated. Meiosis was similar in the shrubs. At first metaphase there were always a number of univalents outside the plate. Their number may be eight or still more. The forming of some multivalents made chromosome counts difficult. Rather clear plates in polar view showed about 40 chromosomes (Fig. 39). The observations indicate that the shrubs are pentaploid. This was confirmed through chromosome counts of root plates from two of the shrubs. One had 95 chromosomes; the other shrub was probably also pentaploid, about 90 chromosomes being discerned in the plates. The observations show that the shrubs have arisen through the union of reduced gametes.

Meiosis and formation of macrospores occur when the integument is forming the micropyle, that is, comparatively late. Cuttings with very young catkins kept indoors sometimes furnished the investigation with materials. Here ovaries may increase in size very rapidly; the development of the somatic tissue of the ovules often stops while meiosis is accelerated. Thus one may find the first metaphase in ovules with only a very low integument. I have made similar observations on other *Salices*. It indicates a certain independence of the generative tissue which, however, does not concern its nutrition: in such cases degeneration occurs.

After NILSSON (1953) we repeat the species used in these crosses: *S. purpurea*, *daphnoides*, *aurita*, *repens*, *phylicifolia* ssp. *nigricans*, *phyl. weigeliana*, *viminalis*, *caprea*, *silesiaca*, *aegyptiaca*, *grandifolia*, *gracilistyla*, *cinerea*, and *glabra*. A pentaploid population was the end result of the crosses.

Conclusion. — The crosses between highly complex *Salix* hybrids treated here seem to be of two different kinds. The crosses leading to *S. polygena*, *S. dasycladoides* and to the 10-hybrid have resulted in a low number of shrubs having high chromosome numbers owing to fertilizations of »unreduced» gametes. On the other hand the crosses leading to the 12-hybrid and the 14-hybrid have resulted in a comparatively higher number of shrubs having intermediary chromosome number. Here, only reduced gametes have functioned.

A curious feature is the good chromosome pairing. Through the

cross leading to the 10-hybrid, two *aegyptiaca* and one *silesiaca* genomes are brought together with the chromosomes of *S. polygena*. Presumably the *aegyptiaca* genomes pair through autosyndesis but it is remarkable that all *silesiaca* chromosomes find partners.

The pairing in the 12-hybrid is still more remarkable. A mixed genome composed of chromosomes from two new species, *S. grandiflora* and *S. gracilistyla*, is here introduced. No chromosome doubling has occurred by the production of this hybrid combination. In spite of this the new chromosomes apparently all find partners, a particularly clear demonstration of the homology of the genomes within the genus section *Caprisalix*.

The more irregular meiosis of the 14-hybrid is hardly due to the introduction of the chromosomes of two new species, *S. cinerea* and *S. glabra*. It is rather similar to meiosis in other pentaploid *Salices*.

GENERAL CONCLUSIONS

Hybrids are easily formed in *Salix*, particularly between species with the same chromosome number. Species hybrids with an even number of genomes generally have a regular meiosis. This indicates a similarity or homology of the genomes of different species, their differences being of genic nature. The different genomes within a polyploid species are also homologous. This is seen in hybrids between diploid and hexaploid species as well as between tetraploid and octoploid species which show absence of univalents and regular meiosis. Thus, the different genomes of the polyploid species are inserted in the chain of homologues. The fertility of the species hybrids with regular meiosis is generally rather good, but, as NILSSON has stressed, the vitality of the F_2 generation is lowered, which is the cause of the preservation of the species differences in nature.

There are certain cytogenetical differentiations within *Salix*. In the section *Caprisalix* most species are diploid, but in *Vitisalix* (*Amerina*) species formation has mainly occurred on the tetraploid level, though *S. triandra* is diploid. One indication of a »gap» between these sections is that hybridizations between species belonging to different sections are difficult, only *S. viminalis*×*S. triandra* being comparatively easy to realize. Another indication is the weakened pairing of investigated section hybrids, *S. viminalis*×*S. triandra* and *S. viminalis*×*S. alba*.

Aneuploid shrubs have been found in certain hybridizations but they are ephemeral; no aneuploid *Salix* species has been found. Much in-

dicates the superiority of euploid gametes. British cytologists have reported the haploid numbers 22, 44, and 88, but have stressed that they do not show aneuploidy, being the result of fragmentation of large chromosomes. Instances of a »22-series» have not been met with here.

The haploid chromosome set of *Salix* I am inclined to call the 19-set; and chromosome morphological studies of WILKINSON have shown 2 L+14 M+2 T+1 S to be its most common chromosome composition (compare p. 454).

Certain rather large differences between the 19-set of a polyploid and a related diploid form are reported by WILKINSON. This should indicate large structural changes but the difficulty of analysing plates with a high number of chromosomes must not be underestimated. Evidence of structural changes in the material investigated by me is furnished by the chromosome bridge formation in the 12-hybrid and at the second anaphase of *viminalis*×*caprea*, and in exceptional cases by unequal bivalents in certain polyploid hybrids. However, the evidence seems small, and the similarity of the different 19-sets shown by WILKINSON is also an indication of the rareness of larger structural changes at species formation in *Salix*.

NILSSON, who has united *S. nigricans* and *S. phylicifolia*, reports the number of *Salix* species native to Sweden to be 23 (the Flora of HOLMBERG has a higher number owing to species splitting). Four are *Vitisalix* species (*triandra*, *alba*, *fragilis*, and *pentandra*) and are not considered further. Among the rest there are five polyploids. Two of the polyploids, *S. cinerea* and *phylicifolia* s.l., have a wide distribution whereas *S. polaris*, *S. glauca* and *S. myrsinitis* are restricted to cold regions. It is, however, an interesting fact that *S. reticulata* and *S. herbacea* (section *Chamaetia*) with a similar occurrence are diploid. The percentage of polyploids in the British flora is higher than in Sweden owing to the occurrence of tetraploid cytotypes of *S. aurita*, *S. caprea* and *S. lapponum*, hitherto not found in Sweden. »Flora of the British isles» contains 20 native species; the polyploid *glauca* is not included, but on the other hand there is the tetraploid *S. atrocinerea*.

In the Central European flora the same *Vitisalix* species occur as in the British and the Swedish flora. Other species (see HEGI) are mostly diploids: there are 17 diploids and only five polyploids while the chromosome numbers of 4—5 species are unknown. In the Japanese flora only two polyploids are hitherto found, *S. phyl. nigricans* and *S. reinii* (*Vitisalix* species not counted).

There exists a kind of *Salices* where polyploidy seems much more

frequent than in the wild species. It is hybrid species or garden species that are more or less anthropochores. Instances of such forms are *S. laurina* (pentaploid), *S. dasyclados* (tetraploid and hexaploid) and *S.*×*stipularis* (hexaploid).

The base number 19 of *Salix* indicates that the genus has an alloploid origin. The same base number occurs in the genus *Populus*, of which the investigated species are all diploids though triploid shrubs are in rare cases found in nature. The frequency of intraspecific polyploidy in *Salix* indicates a rather recent formation of new polyploids. Polyploid species are older and may have been established in the regions previously covered by the Pleistocene glaciation (STEBBINS, 1950, p. 358).

Salicaceae has often been considered a very old family, and occurs among the oldest angiospermous fossils. It has often been considered a member of *Amentiferae*. The simplicity of the flowers has, however, also been interpreted as due to an extreme reduction and not to a retention of archaic features (see LAWRENCE, 1951). The embryology of *Salicaceae* shows reduced traits and it must be a derived rather than a primitive member of *Amentiferae* (HÅKANSSON, 1954, 1955). It may also have no connection with this taxon, having perhaps another position in the angiospermous system. The high base number is no doubt derived but may, on the other hand, be very old. The genus *Magnolia* has the same number; its primitiveness is hardly denied. The 19-base has no connection with chromosome numbers found in indubious *Amentiferae*, 8 and 12 being common base numbers. The chromosome morphological differentiation within the 19-set is also a derived trait but it seems rather conservative within the genus and therefore may be rather old, in all cases older than most of the species' differentiation.

Credit must be given to NILSSON for having experimentally treated to its full extent the species problem in *Salix*. He has, however, a negative attitude to evolution. Concerning the highly complex *Salix* hybrids he writes that when genes from so many and so different species are brought together »when in fact the *genus Salix* has been nearly synthesized it might conceivably be expected that evolutionary older characters might be reconstructed, characters typical of the genus as such and, possibly, characters existing at the time of a presumed separation of the two genera. No characters were found, however, which pointed in the direction of *Populus* or any other genus among *Amentiferae* ... Not even the 13-fold hybrid shows anything to surpass the variation within *Salix*. No Protosalix is obtained, not even the slightest indication of such a genus» (1953, p. 1185). It seems an intricate question, how-

ever, whether crossings between recent species could lead to a recapitulation of the phylogeny of a genus.

Several very interesting new *Salix* types have been produced by NILSSON in his extensive hybridization experiments: many show good fertility. Most of them are new polyploids. The formation of »diploid» macrospores and of »diploid» and »triploid» pollen grains was indicated through cytological observations, while the chromosome number of *S. laurina* indicates the functioning of a »tetraploid» male gamete. Fertile *Salix* forms with an intermediate degree of polyploidy are the autoallosyndesis showing hybrids between a diploid and a hexaploid species as well as between a tetraploid and an octoploid species. Instances are the tetraploid *weigeliana* hybrids and the hexaploid *cinerea*×*reinii*.

Very difficult to explain is, however, the hexaploidy of a cross *S. cinerea*×*S. glabra* where a triploid chromosome number was expected. Here, doubling of the chromosomes of the dividing eggcell seems the only possible explanation, although it is very peculiar that such a doubling should have occurred in several eggcells of the same cross.

There is often a selection within the ovary, only or predominantly plants with increased chromosome numbers appearing in the progeny. In certain cases the selection has probably been on the gonic level, for instance, the production of the hexaploid *dasycladoides* may be due to triploid pollen tubes from the diploid parent growing without difficulty in the hexaploid style tissue: this is probably also the cause of the hexaploidy of certain *polygena* shrubs. Perhaps also a selection on the female side may occur in certain very sterile hybrids, a »diploid» gone more easily forming a normal embryo-sac, while the development of »haploid» gones is arrested in a higher percentage (compare p. 461). No doubt there is also selection on the zygotic level: aborting endosperms and embryos are more or less frequent after heterologous pollinations.

Exceptional so-called extravagant forms may, if relatively fertile, be intermediates in the formation of new polyploids consisting of ♂♂ and ♀♀. From the octoploid *superlaurina* comes *dasylaurina* which, it is true, is heptaploid, but proves to be a very vital and fertile population. The high chromosome number cancels the effect of an odd number of genomes. The 10-hybrid, a hexaploid extravagant form, has given rise to the rather numerous 12-hybrid population but in this case only ♀♀ were produced.

The fact that true intraspecific polyploidy occurs in *Salix*, the different cytotypes showing no taxonomical differences, is also illustrated in

the crosses investigated. The *polygena* has been formed with the tetraploid and the hexaploid chromosome number and is thus a parallel to *S. dasyclados* with the chromosome numbers $2n=76$ and $2n=114$. Thus the results of the crosses of NILSSON seem to bring much experimental material, repeating the mode of forming new *Salix* types and *Salix* species in nature.

NILSSON, however, entertains a very gloomy view of the future of his new forms had they appeared under natural conditions. He is of the opinion that they must be rapidly exterminated in nature. His interesting new *Salices* are, thus, interpreted negatively; they are considered as evidences that the formation of new species at the present time is impossible. This, however, is the manner in which he regards the species problem in the whole plant and animal kingdoms (NILSSON, 1953).

SUMMARY

A number of *Salix* hybrids produced by Professor HERIBERT NILSSON have been investigated cytologically.

Regular meiosis and complete pairing are shown by species hybrids with an even number of 19-sets.

Autosyndesis is common. Hybrids from diploid×hexaploid and tetraploid×octoploid crosses show good pairing and regular meiosis.

Also highly complex hybrids have good pairing and regular meiosis. As instances may be cited a diploid 5-hybrid and a tetraploid 12-hybrid, produced through crosses with five, respectively twelve, different species.

Gametes with doubled and trebled chromosomes have functioned in several hybridizations, causing the origin of forms with an increased number of 19-sets. Instances are hexaploid *S. polygena* from a hexaploid×diploid cross (an 8-hybrid), the hexaploid 10-hybrid from a hexaploid×triploid cross, and the octoploid *S. superlaurina* from a pentaploid×hexaploid cross. The good pairing in such cases is less surprising.

Species hybrids with an odd number of 19-sets and aneuploids have a less regular meiosis owing to the presence of a variable number of univalents. The pentaploid 14-hybrid from a tetraploid×hexaploid cross is an instance; it is produced through crossing fourteen different species.

Section hybrids have weaker pairing.

Two hexaploid *S. cinerea* hybrids did not show the meiotic retardation found in triploid and pentaploid *cinerea* hybrids.

The chromosome numbers of some previously not investigated species have been determined.

According to NILSSON, several of the investigated »extravagant forms» and hybrid populations show many or all criteria of a new species but he does not think that they could be established as new species under natural conditions.

KEY TO SOME COMPLEX HYBRIDIZATIONS

S. (*phylicifolia nigricans*×*phyl. weigeliana*) ♀, hexaploid×*S.* (*viminalis*×*caprea*) ♂, diploid=*S. dasycladoides*, hexaploid.

S. [(*purpurea*×*daphnoides*)×*S.* (*aurita*×*repens*)] ♀, diploid×*S. dasycladoides* ♂, hexaploid = *S. polygena* A, tetraploid, and B, probably pentaploid.

S. dasycladoides ♀, hexaploid×*S.* [(*purpurea*×*daphnoides*)×(*aurita*×*repens*)] ♂, diploid=*S. polygena* D and E, hexaploids.

S. polygena D ♀, hexaploid×*S.* (*silesiaca*×*aegyptiaca*) ♂, triploid= 10-hybrid, hexaploid.

S. (*grandifolia*×*gracilistyla*) ♀, diploid×10-hybrid ♂, hexaploid=12-hybrid, tetraploid.

12-hybrid ♀, tetraploid×*S.* (*cinerea*×*glabra*) ♂, hexaploid = 14-hybrid, pentaploid.

S. laurina ♀, pentaploid×*S. phylicifolia weigeliana* ♂, hexaploid=♂-*laurina*, about pentaploid, *S. superlaurina*, octoploid.

S. superlaurina ♀, octoploid×*S. dasycladoides* ♂, hexaploid=*S. dasylaurina*, heptaploid.

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THE PIGMENT CHARACTERISTICS OF CHLOROPHYLL MUTATIONS IN BARLEY

By *NILS NYBOM*
INSTITUTE OF GENETICS, LUND, SWEDEN

I. INTRODUCTION

GENES or factors controlling the pigment contents or the colour pattern of plants have been studied since the very first years of Mendelism, and were discussed in the now classical papers by, among others, CORRENS, BAUR and DE VRIES. They appeared in almost all diploid and allogamous species after selfing and have also been observed in autogamous species as mutations, spontaneous or induced. For the older literature the reader is referred to the indispensable list of DE HAAN, 1933.

In experimental mutation research the chlorophyll mutants were first and extensively used by STADLER (1928 and later) and have since then played a great rôle as test mutations. They constitute in this respect a parallel to the recessive lethals of *Drosophila* being perhaps to a considerable extent genetically homologous. Examples of the recent use of chlorophyll mutations are given by, *e. g.*, CALDECOTT, BEARD and GARDNER, 1954; EHRENBERG and NYBOM, 1954, and GUSTAFSSON, 1955.

On starting his mutation experiments, GUSTAFSSON also made use of the chlorophyll mutations and worked out a detailed classification scheme (*e. g.*, GUSTAFSSON, 1940), which was subsequently adopted by other workers. HOLM has described this scheme in a recent paper (1954) and has also given some data on the general properties of the chlorophyll mutants. About 50 % of all chlorophyll-deficient mutations in barley are white (*albina*), about 30 % light green (*viridis*), 5 % yellow (*xantha*) and the rest streaked or patterned in various ways.

Some results pointing to the applicability of the chlorophyll mutations for illustrating the important question of differential (or directed) mutability are given by GUSTAFSSON and NYBOM, 1949; D'AMATO, 1950; HAYDEN and SMITH, 1949; MAC KEY, 1954 and EHRENBERG, 1955. Also in our present work, dealing among other things with the linear energy transfer (ionization density) of various radiations and its consequences

for induced mutating, chlorophyll mutations are relatively useful. Data suggest that the different linear energy transfers of X-rays and neutrons bring about genetical effects, differing not only quantitatively but also qualitatively (*cf.* EHRENBERG, 1955).

The spontaneous chlorophyll mutation frequency in barley lies around 0,1—0,2 %, counted as heterozygous spikes in greenhouse analyses.

This spontaneous frequency corresponds to the effect of *circa* 100 r X-rays or 5 rep neutrons, if using the mutation coefficients of EHRENBERG and NYBOM, 1954. Should one estimate that there are a few hundred loci in barley, controlling the chlorophyll synthesis or the plastid properties (which seems reasonable from genetical evidence), the mutation coefficient *per locus and r-unit* will be somewhere around $5 \cdot 10^{-6}$. This figure is in good agreement with corresponding estimates in other materials as widely differing as viruses and bacteria ($0{,}5—3{,}7 \cdot 10^{-6}$; GOWEN, 1941), *Drosophila* ($1{,}5—6 \cdot 10^{-6}$; ALEXANDER, 1954) and mice ($25 \cdot 10^{-6}$; RUSSELL, 1951). This agreement, together with genetical and other evidence, *e.g.*, the possibility to differentiate between chromosome disturbances and chlorophyll mutations (*cf.* CALDECOTT and SMITH, 1952 and also EHRENBERG and NYBOM, 1954, Table 3), support the use of these chlorophyll mutations as a criterion for induced »factor mutations» as different from chromosome aberrations.

Even if principal differences are difficult to uphold these mutations differ quantitatively from the post-meiotically induced »losses of specific alleles» in maize, which seem to appear in a far higher frequency (at least after high dose-rates) and which seem to consist largely of »chromosomal alterations» as shown by DOLLINGER, 1954.

I believe this is the reason for the dose-rate threshold found by SINGLETON, 1954, for these losses. In our first experiments with chronic γ-irradiation we have not obtained the sigmoid curve of SINGLETON, but rather a linear dependence on the daily γ-dose. Already after 20 r/day the mutation frequency is raised 10—15 times over the spontaneous rate.

The works to be reported in this paper have been carried out in collaboration with other members of the Swedish »Group for Theoretical and Applied Mutation Research in Plants».

II. METHODS OF PIGMENT ANALYSIS

For the classification of irradiated materials, as also for studies of the general properties of the individual mutants, one is often in need of easy and rapid methods for estimating the colour grade or pigment content. Ever since the Swedish mutation works started we have used the French colour standard »Code des Couleurs» (abbreviated C. d. C.) for the classification of chlorophyll mutants. It is comparatively well suited for this purpose (much better than several other more modern »colour charts» that have been tested) but it has some very obvious limitations. Thus it is difficult to classify the normal type with it and also the light green mutants, which come close to the normal type (the large group with the C. d. C.-number 276 in Table 3). Moreover, the colour numbers, of course, say nothing of the pigment composition of the plants.

During the last year we spent some time in elaborating easy and rapid methods for the determination of the pigment content of single leaves or parts of leaves. One such method worked out in collaboration with HOLM is described in detail by him (HOLM, 1954). This method is, in principal, similar to that of COMAR and ZSCHEILE, 1942. It is different in that the first acetone extract is read directly on the spectrophotometer without washing or bringing over into ether. Also in some other respects the method has been made more suitable for small leaf samples of about 25—100 mg. Chlorophylls *a* and *b* are determined separately, whereas the yellow pigments are obtained together.

The procedure is briefly as follows (*cf.* also HOLM, 1954). The leaf is ground in a mortar with acetone but without sand. The extract is diluted to 5 or 10 ml in a graduated cylinder and filtered directly down into a 1-cm cuvette. The extract is read at the following wave lengths: 440,5, 644, and 662 mμ. From the extinction values at these wave lengths the concentrations of chlorophylls *a* and *b*, as well as that of the carotenoids (*c*), are calculated according to the following formulae:

$$C_a = 9{,}78 \cdot E_{662} - 0{,}99 \cdot E_{644};$$
$$C_b = 21{,}4 \cdot E_{644} - 4{,}65 \cdot E_{662};$$
$$C_c = 4{,}69 \cdot E_{440.5} - C_{(a+b)} \cdot 0{,}268.$$

The results are obtained as μg pigments per ml extract.

If many analyses are to be carried out, the calculations are made substantially quicker by using the view-line nomograph in Fig. 1. It is used as follows: a ruler (transparent ruler with a fine line) is placed so as to connect the E-values for 644 and 662 mμ (L or R scales are used

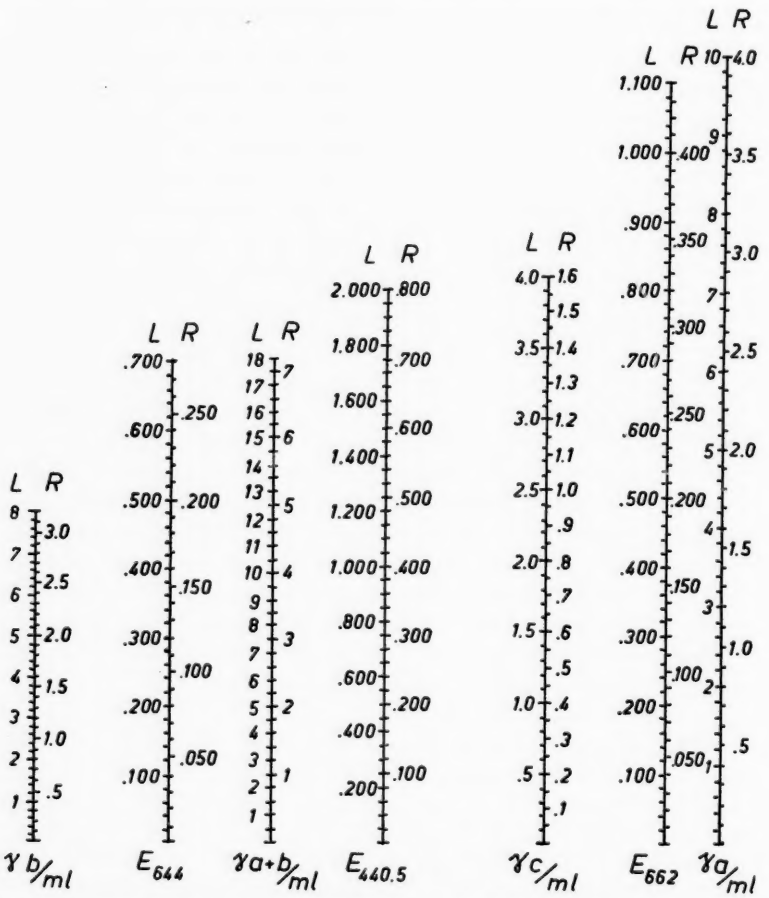

Fig. 1. Nomograph for the easy computation of the pigment content of a solution from the extinction measurements.

according to the concentration range used), after which the concentrations of chlorophylls *b* and *a*, respectively, may be conveniently read at the two extreme scales. The total chlorophyll content $(a+b)$ is also read, and the ruler is then moved to connect this $(a+b)$-value with the E-value at 440,5. The carotenoid content may then be read on the last scale. All concentrations are in μg pigment per ml acetone extract.

The nomograph is also of interest because it illustrates — graphically

— the order of magnitude of the errors in the obtained values that can be expected to result from errors in the extinction values used. An error at 644 will be perceptible almost exclusively in the chlorophyll *b*-content, whereas an error at 662 essentially interferes with the *a*-content. It is also seen that the relative order of magnitude of the errors met with is just slightly larger than those of the E-values themselves. But an error in the extinction at 440,5 mμ will result in an error more than twice as large in the carotenoid content.

Technical remarks. — Concerning the spectrophotometrical part of the analysis the method is certainly accurate enough for most serial work. This is shown, *e. g.*, by the dilution experiments given by HOLM. The biological variation between different samples or even between different parts of the same leaf is of a much larger order of size than the errors inherent in the spectrophotometrical procedure. A trace of $MgCO_3$ should be added before the grinding. When grinding it is important to have enough acetone to prevent pigments from drying on the walls of the mortar. All work is carried out in weak artificial light (tungsten). As spectrophotometer we have used a Hilger Uvispek with very good results. The spectral band width used was 5 Å. It is essential for the separation of the chlorophyll components to use an instrument with narrow band and low stray light contamination. To gain greater accuracy it may be advantageous for the user to construct his own nomograph in a larger scale on mm-paper. The respective height of the scales will then be, *e. g.*, 119,4; 175,0; 176,2; 200,0; 204,9; 275,0; and 284,5 mm, and the distance between the scales: 50,0; 36,1; 41,3; 58,0; 44,6; and 20,3 mm.

III. REFLECTION PHOTOMETRY

In spite of the rapidity of the method just described it may be too slow and cumbersome in larger experiments where one has to classify hundreds of specimens on the same day. Therefore, a still simpler and more rapid method for estimating the pigment content of single leaves has been tested, *viz.*, reflection photometry.

Attachments for diffuse reflection photometry may be obtained for some commercial spectrophotometers but are usually not suitable for measurements on biological objects (the one for Uvispek, *e. g.*, requires a flat, round sample, 0,75 inch in diameter).

To meet these special requirements I have constructed a simple reflection photometer of a new type, shown schematically in Fig. 2. This photometer uses the ocular comparation principle. The leaf, which lies on a glass plate, is illuminated from above, being viewed against a background which is also illuminated. The illumination of the leaf and the background, respectively, may be varied so that they coincide in brightness.

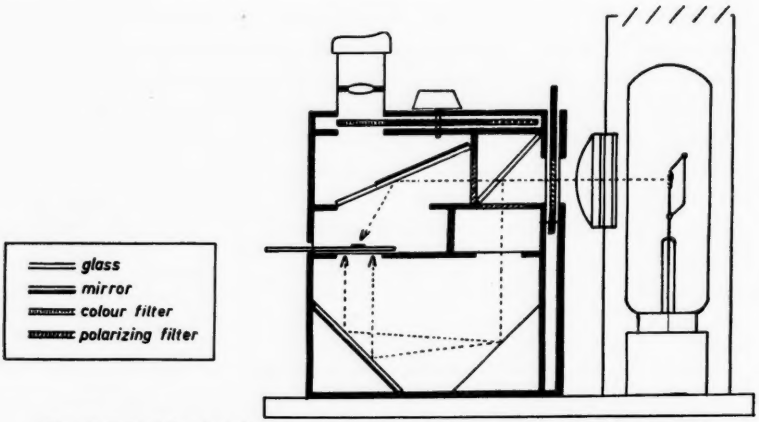

Fig. 2. Principle sketch of the reflection photometer described in the text.

The light, coming from a 250-watt projection lamp, first passes a pair of heat filters and a condensor. It is then split up by a clear glass plate put in 45° angle. Most of the light passes through, is polarized by a polarizing filter, and finally falls on the leaf.

Part of the light is reflected by the glass plate, is polarized by another filter in a plane opposite to the first, and illuminates a piece of white cardboard, acting as a background to the leaf.

A third polarizing filter is made to turn, so that its polarizing plane in its extreme positions coincides with one or other of the first two filters. This arrangement has the effect of the background light being automatically reduced as the light on the leaf is increased, and *vice versa*. In those positions, when the illumination balance with the background is obtained, a light leaf is relatively weakly illuminated, whereas a dark leaf will get much more light. The sensitivity of the instrument is thereby considerably increased, and the scale measuring the angle of the movable filter happens to be almost linearly dependent on the light extinction of the sample.

The instrument has been calibrated with the aid of paper samples with known light reflection. The revolving filter (it is in fact rectangular and does not turn around its centre but around a nail placed close to the midpoint of one of its long sides) is graduated in extinction, *i. e.*: $E = \log_{10} \frac{I_o}{I}$, where I_o = the incident light and I = the reflected ($E = 1{,}1$ means about 8 % reflected light, which corresponds to the normal type

in the red or the blue region, whereas a quite white leaf gives E=0,3, meaning 50 % reflected light).

I am ignorant as to whether this photometer principle has been employed before. It may have been, because it is so simple. The instrument contains no »critical» details and is, therefore, fairly easy to build. With minor changes the same principle could be also used for reflection measurements on other surfaces or bodies of various forms and dimensions.

It is possible to estimate the colour intensity of so small bodies or surfaces that closer analysis with other means seems difficult or impossible. The accuracy of a measurement is good, considering the variation between similar biological objects and also the errors due to differences in surface textures, plastid conditions, water content, *etc.*

For pigment estimations on green leaves the photometer has been provided with two, easily exchangeable, metal interference filters (BAUSCH and LOMB) with transmission maxima at 450 and 640 mμ, respectively.

As is clear from the description of the photometer the light falling upon the leaf is polarized. This is employed in order to reduce the glare due to the parallel surface pattern of a grass leaf. If the leaf is placed with its axis parallel with the incident light this glare is reduced to a minimum. Difficulties from differences in surface texture between the object and the background sometimes appear but may be reduced by adjusting the viewing lens slightly out of focus.

The construction of the instrument has been greatly facilitated by the interest shown by Messrs. A. PREISLER and A. ROTH of The Preisler's Optical Institute, Malmö.

IV. PIGMENT CONTENT AND LIGHT REFLECTION

A number of experiments have been carried out in order to test the applicability of reflection measurements to the estimation of pigment contents of leaves. Normal barley leaves were used as well as a material consisting of different cases of chlorophyll mutations. To increase the variation in pigment content these mutants were cultivated under very different conditions both concerning light (summer sun—artificial light) and temperature (5—30° C.). The various symbols of the diagrams in Figs. 3 and 4 refer to various such cultivation experiments.

As to the chlorophyll content, it has been necessary to determine *a*

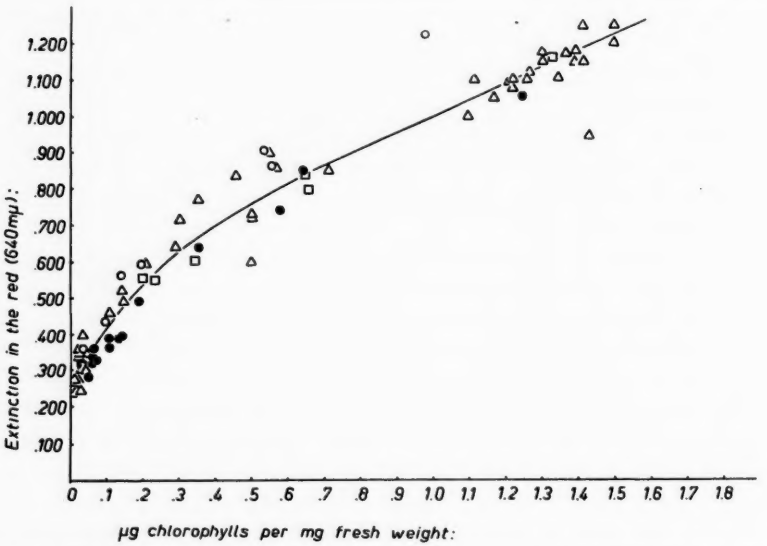

Fig. 3. Diagram showing the connection between content of chlorophylls and the amount of absorbed red light in barley leaves.

and *b* together since the bands given by the available filters were far too broad to allow of any separation of the two components.

As the light absorption of carotenoids is limited to the blue region, the extinction of red light is due exclusively to the chlorophylls, if we disregard the »background» absorption of about 50 % found even in a completely white leaf.

The connection between the light extinction and the total chlorophyll content is shown in Fig. 3. Of course, this curve is valid only for young leaves of barley (5—10 cm long). But it should be possible to make similar calibration curves also for other objects. The pigment content has been calculated as μg pigment per mg fresh weight. No special precautions were taken to keep the water content of the leaves constant. No doubt it varied considerably. Expressing the pigments per surface area would probably give a smoother distribution around the curve.

The swarm of points to the right in the diagram refers to leaves of the normal type, while the rest shows the variation in chlorophyll content among the mutants. The gap between the two categories, the normal leaves and the mutants, is probably artificial and due to the fact that a

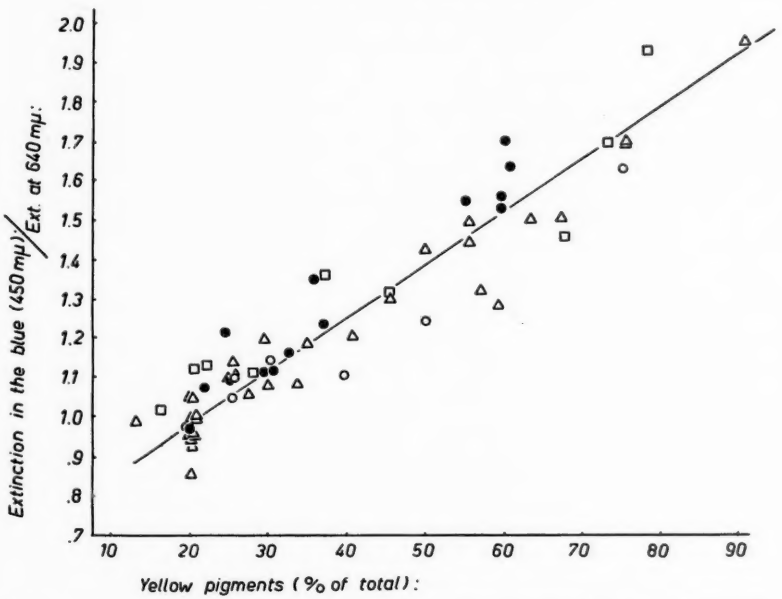

Fig. 4. Diagram showing the connection between the proportion of yellow pigments and the amount of absorbed blue and red light in barley leaves.

certain visual distinctness must be required of a plant in order to be classified as a true mutant. There are certainly many slight *viridis* mutations that pass unnoticed.

In a normal leaf roughly half of the blue light is absorbed by the chlorophylls and the rest by the carotenoids. The quotient between the extinction in the blue and the extinction in the red proved to be the most convenient function of the carotenoid concentration. And it was found best to express the carotenoids as per cent of the total pigment content. In the normal type the percentage of yellow pigments is a very constant figure, about 20 % (*cf.* the vertical row of points at 20 % in Fig. 4).

Fig. 4 shows that the connection between the quotient $E_{450} : E_{640}$ and the percentage of carotenoids is a perfectly linear one in barley leaves. Whether this is a general phenomenon or mere chance in this case seems difficult to say. It is certainly a phenomenon somehow connected with the conditions of the pigments in the living tissue. Densitometric measurements of extracts do not give this straight line.

Fig. 4 also shows that the mutational variation in carotenoid content is a continuous one. Further, it is seen that in all cases (with two uncertain exceptions) the pigment reduction in the first place strikes the chlorophylls, *i. e.*, the percentage of carotenoids in the mutants is always above the 20 % of the normal type.

In none of the mutants chosen for these experiments does the percentage of yellow pigments reach 100 %. Even those mutants which according to Code des Couleurs are classified as *xantha* mutations contain some 10—30 % green pigments (*cf.* also HOLM, 1954).

The considerable variation around the line in Fig. 4 is to be expected because of the fact that the errors in its ordinate are (approximately) equal to the product of those of the two extinction values used.

The pigment content values used on the abscissae of Figs. 3 and 4 have been determined by means of the extraction method described in the preceding paragraph. Errors in these determinations are responsible for a part of the variation in these diagrams.

V. COMPARISON BETWEEN THE EXTRACTION AND THE REFLECTION METHODS

Some comparisons have been made in order to get an idea of the order of size of the errors obtained when the leaf pigments are determined either by means of extraction and transmission spectrophotometry or when they are estimated from measurements of the amounts of reflected light. Thirty-five pairs of similar leaves representing different mutations as well as normal types were analysed with the two methods and their pigment contents were either calculated with the aid of the nomograph in Fig. 1 or read from the curves of Figs. 3 and 4. The arithmetrical difference between the two leaves of each pair was taken as an »error» (biological+technical). The averages of these errors are given in Table 1, distributed into the different concentration ranges. As to the chlorophyll determinations, it is found that at low concentration ranges the reflection method gives almost twice as large an error as the extraction. At higher concentrations, however, the two methods give a more similar result.

The »errors» may seem large, amounting to 10—50 % of the absolute value at high and low concentrations, respectively. It is, however, especially with the reflection method, easy to take an average of 2—3 measurements, and the errors are then at least halved. The difference between the two methods, generally with larger errors for the reflection

TABLE 1. *Comparison between the errors (biological+technical) involved in the two methods for pigment analysis, viz., 1: the extraction method, and 2: the reflection method.*

A total of 35 leaf pairs were analysed.

Microgram chlorophylls (a+b) per milligram leaves:

Concentration range	Average difference between two leaves	
	1: Extraction	2: Reflection
0,0—0,1 µg/mg	0,018	0,033
0,1—0,2	0,038	0,058
0,2—0,4	0,049	0,064
0,4—0,6	0,080	0,093
0,6—1,0	0,107	0,146
1,0—1,5	0,146	0,181

Carotenoids in % of total pigments:

Range of carotenoid percentage	Average difference between two leaves	
	1: Extraction	2: Reflection
10—20 % c.	1,5	4,8
20—30	1,5	5,8
30—50	6,0	7,7
50—70	4,0	13,8
70—100	5,1	18,3

method, is certainly not exclusively of a technical nature, but to some extent a biological one. The leaves are not evenly coloured, and the mutants, especially, may sometimes show a weak mottling. It is then only to be expected that the extraction method, integrating the pigment content of a much larger surface, should give the smallest variation.

This relatively larger inaccuracy of the reflection method is even more pronounced in the estimation of the carotenoid content. In this case, the differences given by the reflection method throughout are more than three times as large. The relative »errors» are, however, somewhat smaller than those of the green pigments, *viz.*, 5—10 % for the extraction method and 20—30 % for the reflection method.

TABLE 2. *The pigment contents corresponding to the most common numbers of »Code des Couleurs», comprising together about 80 % of all mutations.*

C. d. C. Nos.	Colour	Mutation type	Chlorophylls µg/mg fresh weight	Carotenoids in % of all pigments	Cases analysed
171	orange-yellow	f. c.-xantha	26	75	5
196	orange-yellow	xantha	31	71	4
191	orange-yellow	»	39	63	2
236	yellow	»	72	60	2
216	»	»	42	54	8
266	yellow-green	yellow viridis	49	55	5
261	»	yellow viridis	81	55	7
257	»	yellow viridis	215	42	4
286	»	viridis	121	36	2
281	»	»	200	29	6
277	»	»	350	33	2
276	»	»	400—800	20—30	20
278	green	normal type	800—1200	18—20	> 20

f. c. = flame coloured.

In routine analyses it is advisable, due to the relatively large errors, to make a few different measurements on each leaf, changing the position a little, and to use a mental average of them.

VI. THE CHLOROPHYLL MUTATION TYPES

As already mentioned, we use in our research group the Code des Couleurs for classifying the colours of the mutations into *xantha*, *viridis* and others. In Table 2 some analysed samples of chlorophyll mutations are arranged according to their C. d. C.-numbers. The names of the colours are those of the C. d. C., whereas the names of the mutant types follow the rules practised, *viz.*, *xantha* $\leqq 250$ and *viridis* $\geqq 251$. Sometimes mutations with the colours 151—171 are called *flame-coloured xantha* and those with 251—267 *yellow viridis*.

It is generally found that the various mutation types are classified according to the following pigment characteristics:

	μg chlorophyll per g fresh w.:	% carotenoids of all pigments:
flame-coloured xantha	20	80
xantha	40	60
yellow viridis	100	40
viridis	500	30
normal type	1000	20

When the mutants are grouped into such broad classes it appears that the C. d. C. also gives some hints as to their pigment contents. We have seen that it is possible to classify them much closer with photometric methods. One may, however, put the question as to how close it is worth the trouble to determine the pigment content in experiments where light and temperature conditions are not rigidly controlled. Some aspects of this problem are given in the next section.

VII. CHLOROPHYLL DEFICIENCY AND TEMPERATURE

There are in the literature numerous reports showing that the expression of various chlorophyll deficient mutants is highly dependent upon environmental factors, especially of light and temperature, but also of course on edaphic factors.

ÅKERMAN, 1922, and SIRKS, 1929, reported cases where strong light destroyed the pigments of the mutants, whereas HALLQVIST, 1923, and COLLINS, 1927, found that low temperature aggravated chlorophyll deficiencies. The reader is also referred to the valuable list of WEIJER, where several similar cases are reported from the maize literature (WEIJER, 1952).

In connection with the methodological experiments described in paragraph IV, a number of chlorophyll mutants (X_2-progenies after X-ray or neutron irradiations) were cultivated in two identical thermostats with *ca.* 2500 lux; 14 hours per day (fluorescent lamps). One thermostat was kept at 21° and the other at 12° C. The plants were taken for analysis when about 8 cm high, after 8 and 16 days, respectively. The temperatures used correspond fairly well to extreme greenhouse variations.

The results are presented in Table 3. From each case two leaves were analysed. The relations between chlorophylls *a* and *b* show only minor changes in some mutants, whereas the proportion of yellow pigments is highly variable. The most interesting comparison, that between the two temperatures, is facilitated by the relative numbers of the last two

TABLE 3. *Pigment characteristics of some mutants grown at two different temperatures.*

Mutant	No. in Code des Couleurs	The warm thermostat (21° C)						No. in Code des Couleurs	The cool thermostat (12° C.)						Rel. cont. at 21°, % of that at 12°	
		μg per mg fresh weight				a in % of a+b	carot. in % of tot. pigm.		μg per mg fresh weight				a in % of a+b	carot. in % of tot. pigm.		
		Chlorophylls			Caroteno-ids				Chlorophylls			Caroteno-ids				
		a	*b*	*a*+*b*					*a*	*b*	*a*+*b*				Chlor.	Carot.
Normal type:	278	,970	,327	1,297	,337	75	21	278	,972	,305	1,277	,264	76	17	106	86
		,907	,294	1,201	,304	76	20		1,030	,348	1,378	,252	75	16		
52/1101 r. 2136	276 (viridis)	,448	,125	,573	,278	78	33	276 (viridis)	,460	,155	,615	,169	75	22	114	75
		,458	,113	,571	,198	80	26		,500	,181	,681	,192	73	22		
51/1101 r. 324	276 (viridis)	,368	,128	,496	,183	74	27	276 (viridis)	,232	,083	,315	,127	74	29	69	67
		,362	,136	,498	,181	73	27		,252	,120	,372	,145	68	28		
52/1101 r. 1384	277 (viridis)	,284	,055	,339	,160	84	32	257 (yell. vir.)	,220	,048	,268	,135	82	34	67	80
		,303	,057	,360	,174	84	33		,170	,028	,198	,133	86	40		
52/1101 r. 408	281 (viridis)	,141	,056	,197	,079	72	29	171 (f. c.-xa)	,012	,000	,012	,036	100	75	6	43
		,126	,054	,180	,088	70	33		,011	,000	,011	,037	100	77		
52/1101 r. 1637	256 (yell. vir.)	,086	,033	,119	,154	72	56	257 (yell. vir.)	,149	,056	,205	,162	73	44	150	104
		,103	,042	,145	,167	71	54		,142	,046	,188	,171	76	48		
51/1101 r. 477	281 (viridis)	,089	,025	,114	,036	78	24	196 (xantha)	,000	,000	,000	,045	—	100	5	122
		,067	,022	,089	,028	75	24		,010	,000	,010	,033	100	77		
52/1101 r. 1325	236 (xantha)	,067	,021	,088	,136	76	61	171 (f. c.-xa)	,017	,000	,017	,061	100	78	25	55
		,032	,023	,055	,080	58	59		,019	,000	,019	,068	100	78		
52/1101 r. 285	271 (yell. vir.)	,051	,027	,078	,038	65	33	221 (xantha)	,024	,004	,028	,036	86	56	37	89
		,031	,015	,046	,032	67	41		,014	,000	,014	,026	100	65		
51/1101 r. 236	241 (xantha)	,037	,018	,055	,063	67	53	196 (xantha)	,027	,000	,027	,077	100	74	61	134
		,022	,012	,034	,049	65	59		,027	,000	,027	,073	100	73		
52/1101 r. 1676	241 (xantha)	,038	,011	,049	,082	78	63	196 (xantha)	,024	,000	,024	,083	100	77	73	98
		,039	,010	,049	,074	80	60		,039	,008	,047	,068	83	59		

columns, where the pigment contents at the low temperature are expressed in % of that at the higher.

The pigments of the normal type are but little changed. The mutants, however, show marked changes in at least ¾ of the comparisons. Usually the contents have decreased in the cool, but not always, and what is more, the changes in the two kinds of pigment are far from parallel. Summing up, one can say that not two of the mutations studied are alike in their reaction. No less than three out of the seven *viridis* mutations at 21° changed to *xantha* at 12°.

These results are perhaps not unexpected, but to a certain extent rather embarrassing with regard to the mutation experiments. With the scale, in which the scoring of irradiated material has to be carried out, it is very difficult to keep the light and the temperature under exact control. For really critical comparisons one will have to take progenies of the induced mutations and compare them in phytotrons. But if this trouble is undertaken, it might be possible to unveil phenomena which have hitherto remained hidden by these environmental variations.

SUMMARY

Some properties and characteristics of pigment-deficient barley mutations are reviewed. The rules for classifying the mutation types are described as well as methods for determining their pigment contents. A possibly new type of photometer for diffuse light reflection measurements is used for the estimation of the contents of green and yellow pigments in the leaves.

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CYTO-GENETICAL STUDIES IN THE COCHLEARIA OFFICINALIS COMPLEX

By *LISE HÖST SAUNTE*
INSTITUTE OF GENETICS, UNIVERSITY OF COPENHAGEN, DENMARK

TAXONOMICALLY, the arctic-boreal *Cochlearia officinalis* complex has always been difficult to deal with. The taxonomical confusion is amply reflected in the variation of the chromosome numbers which have so far been counted. It is the aim of the present investigation to try to elucidate the taxonomical and evolutionary problems of the complex by a combined cytological, morphological, and genetical approach.

PREVIOUS TAXONOMICAL EVALUATION OF THE COCHLEARIA OFFICINALIS COMPLEX

LINNÉ recognized the following four *Cochlearia* species: the boreal *C. danica* (1753), *C. officinalis* (1753), *C. anglica* (1763), and the arctic *C. groenlandica* (1753). Of these, only *C. danica* has kept its taxonomical position unchallenged.

Concerning the boreal part of the complex, some authors follow LINNÉ and uphold the species *C. anglica* and *C. officinalis*, however supplemented by a number of parallel species of equal taxonomical rank. This is, for instance, the case in one of the most recent British handbooks (CLAPHAM, TUTIN, and WARBURG, 1952) where in addition to *C. officinalis* L., *C. danica* L., and *C. anglica* L. the following species are listed:

C. alpina WATS. (1847) which is probably confined to the mountains of Britain, but may also be found in the mountains of Central Europe.

C. micacea MARSHALL (1894), also a mountain plant reported from Britain and — according to HYLANDER (1941) — from Norway.

C. scotica DRUCE (1928) which seems to be an endemic British species, but closely related to the arctic part of the *C. officinalis* complex.

Other authors have refused to accept these types as more than subspecies; ASCHERSON and GRAEBNER (1898—99), for instance, on the basis of their NE. German material refer to the subspecies *eu-officinalis* (L.) ASCH. and GRAEBN. and *anglica* (L.) ASCH. and GRAEBN.

In his critical treatise on the taxonomical problems of the Scan-

dinavian flora HYLANDER (1941), however, maintains the status of *C. officinalis* L. and *C. danica* L. as species, whereas *C. anglica* L. is given varietal rank only under the name of *C. officinalis* var. *anglica* (L.) ALEF. (1866). Parallel to this variety he recognizes the varieties *integrifolia* (HARTM.) HYL. (1941) and *fenestrata* (R. BR.) HYL. (1941) of which the former — according to HYLANDER (1945) — is synonymous with *C. micacea* MARSHALL, while the latter belongs to the arctic part of the complex.

Furthermore, two types from Poland should be mentioned which have recently been investigated by BAJER (1950, 1951), namely, *C. Tatrae* FRÖHL. (1937), a mountain plant, and *C. polonica* BORB. (1895) which is confined to the sand covered desert planes in S. Poland.

When we turn to the arctic and the subarctic part of the *C. officinalis* complex the picture is much the same. Apart from LINNÉ's *C. groenlandica* the following species are listed in the literature:

C. fenestrata R. BR. (1819), collected on the coasts of Baffin's Bay in Arctic Canada. According to HYLANDER (1941), it also occurs in Scandinavia.

C. lenensis ADAMS (1821), found at the River Lena in NE. Siberia.

C. oblongifolia DC. (1821), from St. Paul's Island off the coast of the Eastern Taimyr Peninsula in W. Siberia.

C. arctica SCHLECHT. (1821), from Northern Siberia.

C. sessilifolia ROLLINS (1941), from Kodiak Island, Alaska.

Furthermore, it would seem natural to include the already mentioned *C. scotica* DRUCE (1928) from Scotland in this list.

As mentioned above, HYLANDER regarded *C. fenestrata* as a variety, and before him LANGE (1887) divided *C. groenlandica* L. into two varieties, *minor* and *oblongifolia* — while he has *C. fenestrata* R. BR. as a distinct species — and GELERT (ANDERSSON and HESSELMAN, 1900) subdivided *C. officinalis* L. into four forms: f. *typica*, which, however, belongs to the boreal part of the complex, and the three arctic forms, f. *groenlandica* (L.) GEL., f. *oblongifolia* (DC.) GEL., and f. *arctica* (SCHLECHT.) GEL.

HULTÉN (1928) concludes that »it seems most convenient to regard the circumpolar *Cochlearia* forms as belonging to one species having several geographical races» and regards the arctic types as subspecies of *C. officinalis*, namely, ssp. *oblongifolia* (DC.) HULT. and ssp. *arctica* (SCHLECHT.) HULT. *C. sessilifolia* is considered by HULTÉN (1945) to be a variety of *C. officinalis arctica*. In that paper HULTÉN also considers

C. groenlandica to be closely related to *C. officinalis arctica*, and *C. anglica* to be doubtfully distinct from *C. officinalis*.

PREVIOUS CYTOLOGICAL INVESTIGATIONS

A list of the chromosome numbers that have so far been counted is given in Table 1.

In addition, the chromosome numbers published by A. and D. LÖVE (1948) on the basis of information placed at their disposal by SÖRENSEN and WESTERGÅRD is given in Table 2.

The present author is much indebted to SÖRENSEN and WESTERGÅRD for their kind permission to use this material which will be discussed below.

TABLE 1. *Chromosome numbers counted by previous authors.*

Author	Species	Locality	2*n*
CRANE and GAIRDNER, 1923	*C. danica*	St. David's off the SW. coast of Wales	42
» » » »	*C. officinalis*	St. David's off the SW. coast of Wales	28
» » » »	*C. anglica*	Hayling Island, Hants.	49–50, 36–44
» » » »	*C. alpina*	?	28
» » » »	*C. micacea*	?	34–36
MATSUURO and SUTO, 1935	*C. oblongifolia*	Akkesi, prov. Kusiro	14
BÖCHER, 1938	*C. officinalis* var. *alpina*	The Faroes	24
GAIRDNER (in MAUDE, 1939)	*C. scotica*	?	14
FLOVIK, 1940	*C. officinalis* var. *groenlandica*	Spitzbergen	14
BAJER, 1950	*C. polonica*	?	36
» »	*C. Tatrae*	?	42
BÖCHER and LARSEN, 1950	*C. officinalis* incl. var. *groenlandica* et var. *arctica*	W. and SW. Greenland	14
HOLMEN, 1952	*C. officinalis* var. *groenlandica*	Pearyland, N. Greenland	14 + f

TABLE 2. *Chromosome numbers counted by* SÖRENSEN *and* WESTERGÅRD.

Species	2*n*
C. officinalis	24 + B
C. anglica	48 + B
Cochleariae from Greenland	14

TABLE 3. *Chromosome numbers counted by the author.*

Collector	Species	Locality	2*n*	Number of samples
K. HOLMEN	*C. groenlandica* s. l.	Pearyland, N. Greenland	14	1
»	»	Brönlund Fj., N. Greenland 82° 12′ N. 32° W.	14	1
H. MATHIESEN	»	Wollaston Forland, NE. Greenland 74° 28′ N. 20° 35′W.	14	1
T. W. BÖCHER	»	Sukkertoppen, SW. Greenland	14	1
C. A. JÖRGENSEN	»	Ikorfat, NW. Greenland	14	14
»	»	Kuk, NW. Greenland	14	2
»	»	Tugtugtaq Island, Julianehåb, S. Greenland	14	1
»	»	Narssak, Julianehåb, S. Greenland	14	1
»	»	Lichtenau-vf., S. Greenland	14	1
G. GUDJÓNSSON	»	Naustvik, 'Arnestr., NW. Iceland	14	1
»	»	Naustvik, Reykjafjördur, NW. Iceland	14	1
TH. SÖRENSEN	*C. danica*	Fugleholm, Helnæs, Fyn	42	1
»	»	Rörvig, Sjælland	42	1
AUTHOR	»	Kongelunden, Amager	42	1
M. WESTERGÅRD	»	Glænö	42	1
TH. SÖRENSEN	»	Roden, Lolland	42	1
C. A. JÖRGENSEN	»	Hammeren, Bornholm	42	1
SV. E. OLSEN	*C. officinalis*	Talvik, N. Norway	24	1
A. NYGREN	»	Alta, N. Norway	24	1
T. W. BÖCHER	»	Teesdale, Durham, N. England	24	1
K. JESSEN	»	Ben Bulben, Co. Sligo, NW. Ireland	24	1
»	»	Black Head, Co. Clare, W. Ireland	24	9
»	»	Lauragh, Kenmare, SW. Ireland	24	3
TH. SÖRENSEN	»	Svinklöv kridtklint, Jylland	24	1
»	»	Klitgårde, Ålborg, Jylland	24	2
»	»	Eiby strand, Hornsherred, Sjælland	24	1
M. WESTERGÅRD	»	Boserup, Sjælland	24	1
AUTHOR	*C. anglica*	Kongelunden, Amager	48	1
T. CHRISTENSEN	»	Skælskör Nor, Sjælland	48	1
TH. SÖRENSEN	»	Stignæs, SW. Sjælland	48	2
»	»	Roden, Lolland	48	1
»	*C.* »*anglica*»	Gylling Næs, Horsens Fjord, Jylland	36	1

Unfortunately, samples from Barter Island, N. coast of Alaska, and from St. Paul Island, Pribilof Isls. collected by LAURENT-CHRISTENSEN and POLUNIN, respectively, did not germinate.

Most of the 24- and 48-chromosome types have in addition a number of B-chromosomes varying from 1 to 6 (see p. 512).

OWN INVESTIGATIONS

The chromosome numbers counted by the author are in many ways in accordance with the earlier countings, but give a more simplified picture of the situation.

The material consisted mostly of seeds collected in the Arctic and in the Scandinavian countries. The seeds were germinated and the plants cultivated in the Botanical Garden in Copenhagen.

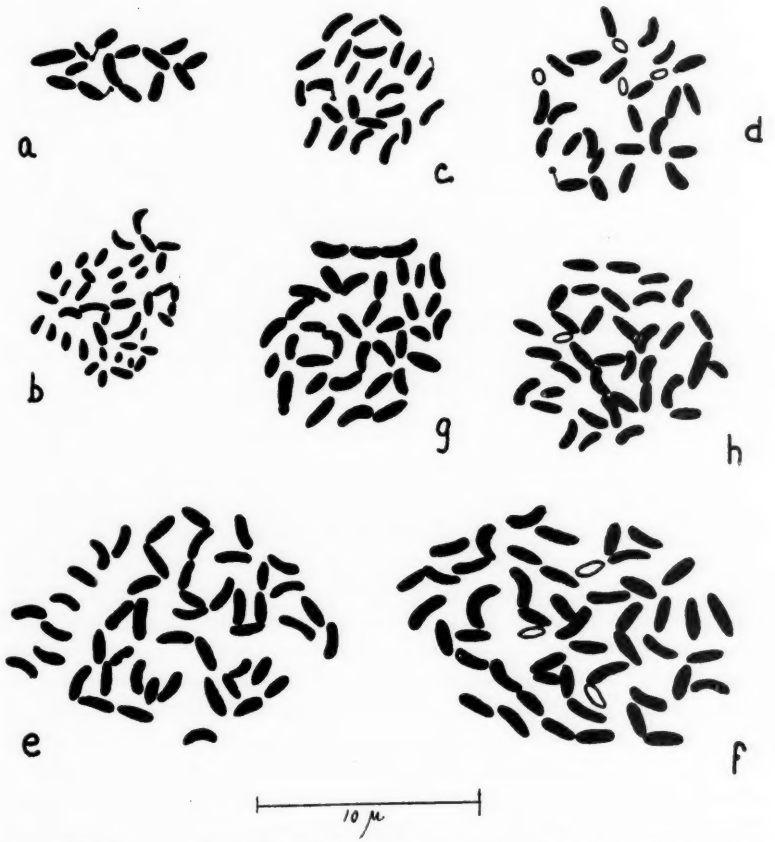

Fig. 1. Mitosis. — *a.* Plant from Kuk with $2n=14$. — *b.* Plant from Stignæs with $2n=42$. — *c.* Plant from Eiby strand with $2n=24$. — *d.* Plant from Alta with $2n=24+4$ B. — *e.* and *f.* Plant from Stignæs with $2n=48$ and $2n=48+3$ B. — *g* and *h.* Plant from Gylling Næs with $2n=36$ and $2n=36+1$ B.

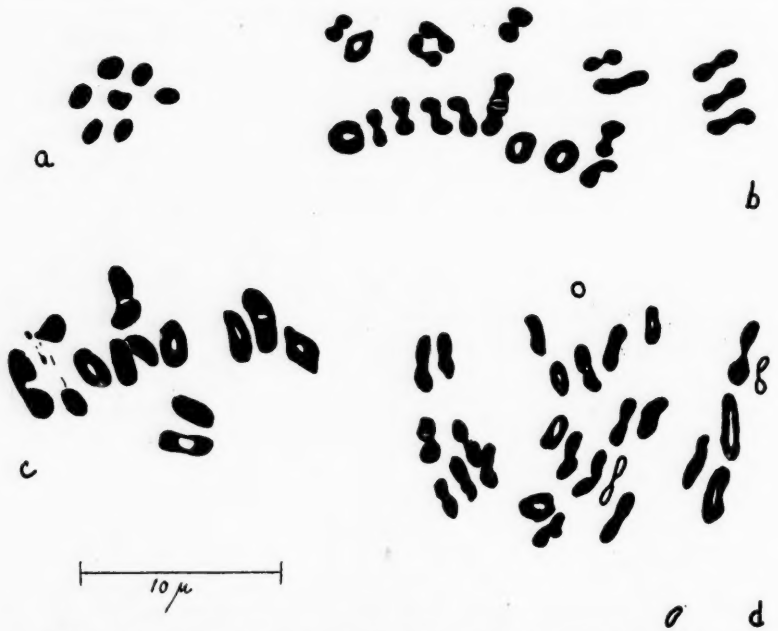

Fig. 2. Meiosis. — *a*. Plant from Greenland (after one of WESTERGÅRD's slides) with $2n=14$. — *b*. Plant from Fugleholm with $2n=42$. — *c*. Plant from Lauragh with $2n=24+2$ B. — *d*. Plant from Stignæs with $2n=48+6$ B.

The somatic chromosome numbers were counted in root tips after fixation in Lewitsky, embedding in paraffin, sectioning (7—10 μ), and staining with crystal violet. Whenever possible the counting was based on root tips from ten plants from each seed sample. The meiotic chromosomes were counted in PMC in squash preparations stained with carmine or orcein, in some cases a prefixation with Carnoy was used. The drawings of the chromosomes and of the pollen grains were made by means of a camera lucida.

The results of the chromosome countings are given in Table 3.

The existence of two basic numbers was established, represented by a 7-series of diploids and hexaploids, and a 12- (or 6-) series of diploids (tetraploids) and tetraploids (octoploids).

The 14-chromosome complex. — The high-arctic species collected in Greenland and Iceland — 25 samples — all have the diploid number $2n=14$. The chromosomes in mitosis (Fig. 1 *a*) and meiosis (Fig. 2 *a*)

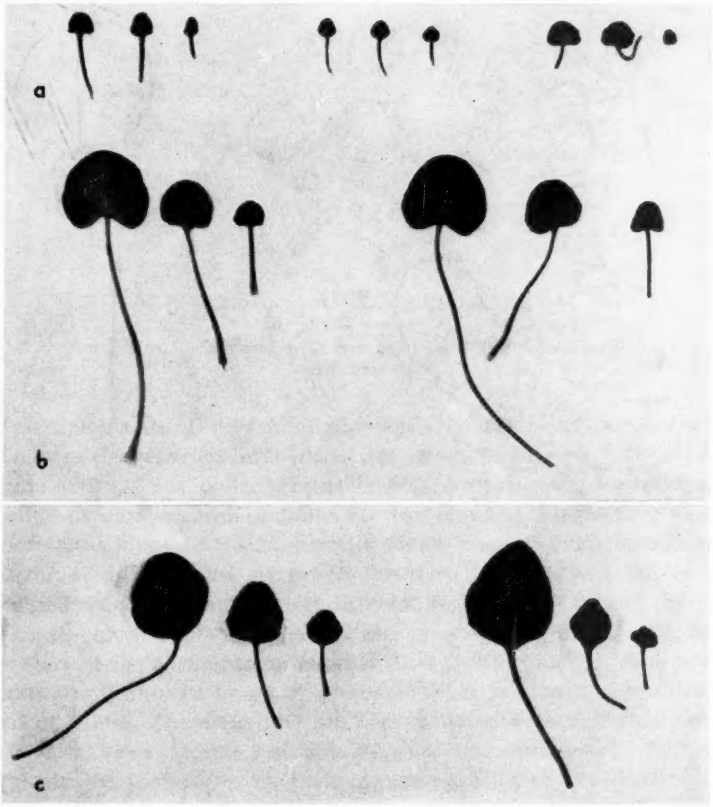

Fig. 3. Basal leaves. — *a.* 3 plants with $2n=14$ (from Ikorfat, Pearyland, and Naustvik). — *b.* 2 plants with $2n=24$ (from Eiby strand and Klitgårde). — *c.* 2 plants with $2n=48$ (from Roden and Stignæs). — (Photo by A. ÖYE.)

are very uniform, their length being about 2 μ. Satellites were not always easy to observe, but may be seen clearly in the plate depicted in Fig. 1 *a*.

These countings are in accordance with the chromosome numbers found by MATSUURO and SUTO in *C. oblongifolia* from N. Japan, by GAIRDNER in *C. scotica* from the Northern parts of the British Isles, by FLOVIK in *C. officinalis* var. *groenlandica* from Spitzbergen, by SÖRENSEN and WESTERGÅRD in material from Greenland, by BÖCHER and LARSEN in *C. officinalis* incl. var. *groenlandica* and var. *arctica* from

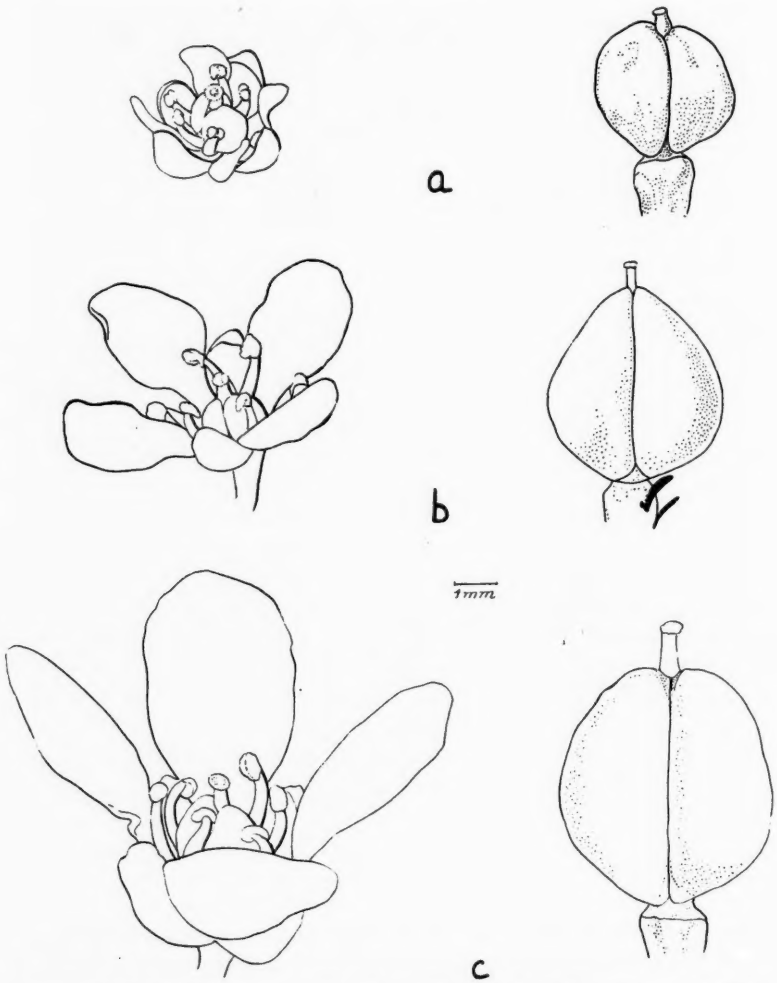

Fig. 4. Flowers and siliculae. — *a*. Plant from Tugtugtaqöen with $2n=14$. — *b*. Plant from Klitgårde with $2n=24$. — *c*. Plant from Stignæs with $2n=48$. — (Drawings by J. TESCH.)

W. and SW. Greenland, and by HOLMEN in *C. officinalis* var. *groenlandica* from Peary Land. HOLMEN is the only author who has found B-chromosomes in these arctic types.

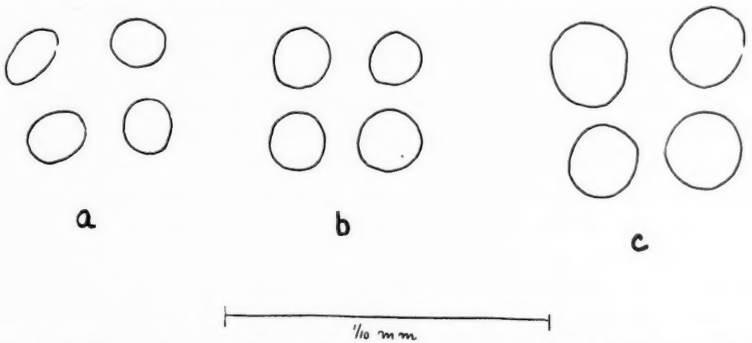

Fig. 5. Pollen grains. — *a*. Plant with $2n=14$. — *b*. Plant with $2n=24$. — *c*. Plant with $2n=48$.

The diploids are all very small plants highly variable in morphological characters (leaves from three plants are shown in Fig. 3 *a*, a flower and silicula in Fig. 4 *a*, pollen grains in Fig. 5 *a*). It was, however, not possible to analyse and evaluate the taxonomical significance of the variation, as many of the high-arctic plants were difficult to cultivate, and the seeds produced were very poor, so that it could not be ascertained whether samples from different regions were intersterile.

The 42-chromosome complex. — The investigation of 6 Danish samples showed the chromosome number $2n=42$, the same number which CRANE and GAIRDNER found in *C. danica* from St. David's off the SW. coast of Wales. These samples are very uniform both morphologically and cytologically. Morphologically, they represent the true *C. danica* L. Cytologically, they differ by their idiograms from the 14-chromosome species, and from all other types investigated (Figs. 1 *b* and 2 *b*). Owing to the small size of the chromosomes it is not possible to give a complete idiogram, but in the best cells there seem to be 14 large chromosomes, 14 medium-sized, and 14 small ones. It should be added that the size of the smallest chromosomes is approximately the same as that of the B-chromosomes in the series with 12 as basic number which will be discussed below.

The 24-chromosome complex. — The 21 samples in this group have been collected in N. Norway, Denmark, England, and Ireland.

Morphologically, although a certain variation is present, they are typical *C. officinalis* (typical reniform-cordate basal leaves are shown in Fig. 3 *b*, flower and the rather small spherical silicula in Fig. 4 *b*, and pollen grains in Fig. 5 *b*). This group has 24 chromosomes of equal size,

about 2 μ long (Fig. 1 *c*), but in many cases B-chromosomes are present (Figs. 1 *d* and 2 *c*). The B-chromosomes will be discussed in a later paragraph.

These results are in accordance with those of BÖCHER who found $2n=24$ in *C. officinalis* var. *alpina* from the Faroes and those of SÖRENSEN and WESTERGÅRD who found $2n=24+B$ in their *C. officinalis* material. The accordance with CRANE and GAIRDNER's results may seem less apparent, but in one of their illustrations it is possible to distinguish 24 chromosomes of normal size and 4 minor ones, so it would seem legitimate to interpret their result — $2n=28$ — as $2n=24+4$ B. This would corroborate the opinion that $2n=24$ is the true chromosome number of *C. officinalis* s. str.

The 48-chromosome complex. — 5 Danish samples identified by their morphological characters as *C. anglica* all have the chromosome number $2n=48$. (The obovate-cuneate basal leaves are shown in Fig. 3 *c*, the large flower and the large swollen silicula with its narrow septum in Fig. 4 *c*, and pollen grains in Fig. 5 *c*.) Both by chromosome morphology (Fig. 1 *e*) and the presence of B-chromosomes (Fig. *1 f*) they resemble the 24-chromosome group very closely. The countings are in accordance with those of SÖRENSEN and WESTERGÅRD and also with those of CRANE and GAIRDNER from Hayling Island, Hants., if we interpret their number of $2n=49$—50 as $2n=48+1$—2 B which seems justified by their failure to establish any definite number. Evidently the true number of this type is $2n=48$.

Meiosis (Fig. 2) has been studied in all these types and only regular bivalent and no polyvalent associations have been observed. The behaviour of the B-chromosomes will be discussed below.

The 36-chromosome complex. — Only one sample from Gylling Næs collected by SÖRENSEN in 1948 has the chromosome number $2n=36$. The chromosomes were like the ones found in the 24- and 48-chromosome groups, and the presence of B-chromosomes was demonstrated (Figs. 1 *g* and *h*). Meiosis is somewhat less regular than in the other groups; the fertility is good. The ten plants from this sample were very variable, although in most characters somewhat closer to *C. anglica* than to the *officinalis* type.

Concerning this 36-chromosome type two explanations are possible: it is either a true species, hitherto overlooked in Denmark, or a hybrid between plants with $2n=24$ and plants with $2n=48$. The first of these explanations could not be excluded beforehand as true hexaploids exist and have been described from Britain (*C. micacea*) and from Poland

(*C. polonica*) (Table 1). Furthermore, CRANE and GAIRDNER have reported $2n=36$—44 for some of their *C. anglica* material. In order to try to solve the problem the locality was visited in May, 1954, and the population proved to be a highly complex hybrid one.

This hybrid swarm containing forms that vary from pure *C. officinalis* to pure *C. anglica* is now being investigated parallel to the investigation of experimentally produced hybrids of the two species. A population found at Knudshoved, Fyn, is most likely of a similar character.

Crossing experiments. — The results of crossing experiments carried out to supplement the cytological investigations are given in Table 4.

Up till now all attempts to cross the $2n=14$ plants into plants with other chromosome numbers have been failures, whereas the crossings between 24- and 42-plants and between 48- and 42-plants were successful. It proved possible to backcross the natural hybrids with 36 chromosomes to either parent, and the hybrid could also be crossed to the 42-chromosome plants.

TABLE 4. *Author's crossing experiments.*

♂ \ ♀	*C. groenlandica* s.l. $2n=14$	*C. officinalis* $2n=24$	*C. »anglica»* $2n=36$	*C. danica* $2n=42$	*C. anglica* $2n=48$
C. groenlandica s.l. $2n=14$		—	(—)	—	—
C. officinalis $2n=24$	(—)		+	—	+
C. »anglica» $2n=36$	(—)	+		○	—
C. danica $2n=42$	○	+	+		+
C. anglica $2n=48$	(—)	+	(—)	○	

\+ The crossing succeeded, the seeds germinated.
(—) » » » , » » did not germinate.
— » » did not succeed.
○ » » was not tried.

Of the seven successful crossings five gave seeds for the raising of F_2 families that are now being investigated. (The seeds from the F_1 generation produced by crossing *C. anglica* $2n=36$ to *C. danica* $2n=42$ and *C. officinalis* $2n=24$ to *C. danica* $2n=42$ did not germinate.)

DISCUSSION

The present investigation has shown the coexistence of two different basic numbers within the genus. A 7-series represented by diploids ($2n=14$) and hexaploids ($2n=42$) and a 6-series represented by tetraploids ($2n=24$), hexaploids ($2n=36$), and octoploids ($2n=48$). The fact that true-breeding species with $2n=36$ (*C. polonica* and *C. micacea*) exist shows that 6 and not 12 is the true basic number. The taxonomical significance of these observations will be discussed for each chromosome number.

The 7-series. — The 42-chromosome species *C. danica* is the only annual plant of those investigated. Cytological as well as morphological evidence point to the conclusion that this species is a distinct and well established one. This evaluation is not invalidated by the fact that it sometimes hybridizes in nature with the 24- and 48-chromosome types and that such hybrids can be produced experimentally. The experimentally produced F_1 generation has a very irregular meiosis, and preliminary observations of the experimentally raised F_2 families show a number of dwarfish, abnormal types with very low fertility or complete sterility.

The chromosome numbers suggest that *C. Tatrae* from Poland might be related to *C. danica*, but the cytological investigation carried out by BAJER demonstrates that the chromosome morphology is not the same in the two species, as the *Tatrae* chromosomes are all of the same size, whereas *C. danica* has chromosomes of three different sizes. (While *C. danica* is the only species in the author's material having chromosomes of different sizes the same is reported by BAJER for *C. polonica*.) FRÖLICH's morphological investigation of 8 *Cochlearia* species also leads to the conclusion that these two species with 42 chromosomes (*C. danica* and *C. Tatrae*) are not closely related.

The arctic-subarctic complex with $2n=14$ is isolated both cytologically and genetically from the other groups. All plants from Greenland have 14 chromosomes, and the same is true for those hitherto investigated from Iceland. Most, or probably all, plants from Greenland may be referred to *C. groenlandica* L., but they may with equal right be grouped

as *C. groenlandica*, *C. oblongifolia*, and intermediate forms. The plants from Iceland are all very variable, and we have not yet been able to classify them with any degree of certainty. In this connection it should be mentioned: (1) that it is very difficult to distinguish between *C. groenlandica*, *C. oblongifolia*, and *C. arctica;* (2) that plants from Greenland developed very poorly in the Botanical Garden; (3) that herbarium material from Iceland is so variable that it seems impossible to determine the question of nomenclature, but (4) that the plants cultivated in Copenhagen from seeds from Iceland seemed to represent *C. oblongifolia*. FLOVIK has $2n=14$ for his *C. officinalis* var. *groenlandica*, and so has GAIRDNER for his *C. scotica*, but the present author has not had the opportunity to compare their material with the arctic collections at her disposal, which makes it difficult or impossible to make conclusions about the possible identity of these types. Geographically completely isolated from these forms is *C. oblongifolia* from N. Japan with $2n=14$. Unfortunately, the chromosomes of *C. arctica* and *C. lenensis* from Siberia and *C. fenestrata* and *C. sessilifolia* from N. America have not been counted. As long as these gaps in our knowledge have not been bridged, it is premature to form any definite opinion concerning the cyto-taxonomical relationship of the circumpolar *Cochleariae*. It seems, however, the most logical to include all the 14-chromosome types into one species: *C. groenlandica* L. of which *C. scotica* and *C. oblongifolia* should be given subspecific or varietal rank. Whether the arctic species whose chromosomes have not been counted should be included in *C. groenlandica* or should be grouped with the following species must, however, await further investigation.

The 6-series. — The *officinalis* group with $2n=24$ and the *anglica* group with $2n=48$ represent two polyploid levels. Among modern taxonomists there is a tendency to give specific rank to such levels. The reason is that polyploidy usually establishes a sterility barrier between the groups so that gene interchange is prevented. It would thus seem natural to place the 24-chromosome type as a separate species under the name of *C. officinalis* L. and the 48-chromosome type as a parallel species under the name of *C. anglica* L. There are, however, some reasons which induce us to hesitate to follow this rule in the present case where we seem to be facing the rather unique situation that differences in ploidy have not established a sterility barrier sufficiently strong to prevent an almost normal gene exchange. For one thing meiosis both in the experimentally produced 36-chromosome hybrids between plants with $2n=24$ and $2n=48$ and in the naturally occurring 36-chromosome

types from Gylling Næs is almost regular; in addition, the hybrids are fully fertile, and the abnormal dwarfish types do not occur in the F_2 families. It may, therefore, be more cautious — at least at the present stage of the investigation — to follow the suggestion of ASCHERSON and GRAEBNER and give the two types subspecific rank for which the proper names should be ssp. *eu-officinalis* (L.) ASCH. and GRAEBN. and ssp. *anglica* (L.) ASCH. and GRAEBN.

It is not possible at the present to express any definite opinion concerning the taxonomical rank of the smaller units within the *officinalis* complex, but if *eu-officinalis* and *anglica* are given subspecific rank it does not seem reasonable to maintain *C. alpina* with 24 chromosomes as a separate species. (BÖCHER gives this number for plants from the Faroes, and the number 28 as given by CRANE and GAIRDNER may with good reason be interpreted as $2n=24+4$ B as in the case of *C. officinalis*.)

Evidently plants with $2n=36$ represent two different categories. In Denmark they represent hybrids between 24-chromosome ssp. *eu-officinalis* and 48-chromosome ssp. *anglica*. In Poland (*C. polonica*) and in Britain (*C. micacea*) true-breeding distinct types seem to occur.

The evolution of the two basic numbers offers a very interesting problem for further studies. This may have been effectuated in two ways: (1) on a diploid level from $2n=14$ to $2n=12$ or *vice versa*, in which case we would venture to postulate the existence of a yet unknown or extinct 12-chromosome diploid species, or (2) on a higher ploidy level. It is a rather astonishing fact that in the 7-chromosome series no types with chromosome numbers to bridge the gap between the arctic 14-chromosome diploids and the boreal 42-chromosome hexaploid *danica* have been found. The very characteristic chromosome idiogram of *danica* makes this problem even more puzzling. In this connection it would be very important to have CRANE and GAIRDNER's above-mentioned counting of the chromosomes of the British *C. alpina* confirmed or invalidated.

The B-chromosomes. — B-chromosomes in varying numbers — from 1 to 6 in the author's material — are frequently found in the *Cochlearia* species with $2n=24$ and $2n=48$, but neither in those with $2n=14$ nor in those with $2n=42$, although HOLMEN found 1 B-chromosome in an arctic plant with $2n=14$ and BAJER reports tiny fragments in *C. Tatrae* with $2n=42$. In meiosis the B-chromosomes show a tendency to separate earlier than the normal chromosomes, as will be seen from the metaphase plates in Fig. 2. Neither in *Cochlearia* nor in other plants has it

been possible to form a definite opinion on the origin and function of the B-chromosomes. One possible explanation concerning the origin of the B-chromosomes in this genus shall be mentioned. In form and size the B-chromosomes correspond to the smallest chromosomes in *C. danica*, and we know that it is possible to get fertile hybrids between *C. danica* and members of the other chromosome groups. It is therefore theoretically possible that the B-chromosomes are the small *danica* chromosomes remaining from earlier crossings. This problem may be solved by crossing B-chromosome types of *C. officinalis* and *anglica* to *danica* and investigate the pairing of the chromosomes in meiosis.

The author owes her sincere thanks to all who have contributed by collecting plants and placing material at her disposal and to Mr. PETER JACOBSEN, M. Sc., who shared the work of cultivating the plants in the Botanical Garden. A special thank is due to Professor TH. SÖRENSEN of the Royal Veterinary and Agricultural College, Copenhagen, for his valuable assistance concerning all taxonomical problems. The author is also indebted to the head of the Institute of Genetics, Professor M. WESTERGÅRD, for guidance and help. The investigation was made possible by financial support from the Carlsberg Foundation, and Dansk Botanisk Rejsefond provided a grant to cover travelling expenses.

SUMMARY

The chromosomes of 59 samples of the *Cochlearia officinalis* complex from Scandinavia, Great Britain, and Greenland have been counted.

All plants from Greenland and Iceland have the mitotic number of 14.

All plants referred to *C. danica* have $2n=42$.

Plants referred to *C. officinalis* always have $2n=24$, whereas all plants referred to *C. anglica* have $2n=48$. These two groups have varying numbers of B-chromosomes.

Several hybrids (Table 4) have been produced and in some cases raised to F_2.

Hybrid swarms with $2n=36$ have been found in nature between plants with $2n=24$ and $2n=48$.

The taxonomical implications of these facts have been discussed and the following suggestions made:

(1) It is suggested that all the 14-chromosome types are included into one species under the name of *C. groenlandica* L.

(2) *C. danica* L. should be maintained as a separate species.

(3) It is suggested to follow ASCHERSON and GRAEBNER and give the *officinalis* and *anglica* types rank as subspecies: *eu-officinalis* (L.) ASCH. and GRAEBN. and *anglica* (L.) ASCH. and GRAEBN.

(4) True-breeding 36-chromosome species occur as endemics in Britain (*C. micacea*) and in Poland (*C. polonica*).

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BRIEF REPORTS

ARNE ROUSI: Cytological observations on the *Ranunculus auricomus* group.

ROZANOVA (1932) was the first to point out that reproduction in the great species group *Ranunculus auricomus* is pseudogamous. The seeds develop apomictically without fertilization, but not without pollination. The same conclusion has been reached by KOCH (1933, 1939) and SÖRENSEN (1938). HÄFLIGER (1943), again, has investigated the formation of the embryo sac and the embryo in the *Ranunculus auricomus* group. The embryo sac arises aposporously, *i. e.*, from a somatic cell of the nucellus. According to him, the embryo as well as the endosperm are formed without fertilization. However, recent (unpublished) results by RUTISHAUSER which concern the chromosome numbers in the endosperm of some *R. auricomus* microspecies (cited by NYGREN, 1954) indicate that the endosperm usually develops only after the central nucleus has been fertilized.

As is usual in apomictic plant groups, *Ranunculus auricomus* falls into a great number of separate and constant forms, so-called microspecies. The taxonomy and distribution of the Finnish forms have been extensively studied by MARKLUND (1939, 1954). MARKLUNDs results are, however, to a great extent unpublished. KOCH (1933, 1939) has used for the microspecies a binomial classification in the manner of sexual species. In contradistinction to this, MARKLUND regards the Finnish forms as subspecies which he unites into four species: *R. auricomus*, *R. fallax*, *R. cassubicus*, and *R. monophyllos*.

Out of the 17 Swiss *auricomus* microspecies investigated by HÄFLIGER (1943) 14 were tetraploid ($2n=32$), one diploid ($2n=16$), one pentaploid ($2n=40$), and one hexaploid ($2n=48$). The chromosome numbers of 24 Finnish microspecies have now been determined. Of these, 22 were tetraploid, one was probably pentaploid (*R. auricomus* ssp. *calvescens*) and one contained both tetraploid and hexaploid forms (*R. auricomus* ssp. *oligandrus*). Neither these nor the other Finnish microspecies are found in Switzerland.

The meiotic divisions in the *Ranunculus auricomus* group previously have been relatively little studied (BÖCHER, 1938; HÄFLIGER, 1943). In the present study the meiosis in the PMC of 11 tetraploid microspecies has been analysed. During the meiotic prophase the chromosomes contract into a group three different times. First, this occurs during zygotene, when a bouquet formation is seen. This happens second time in diplotene, and a third time immediately before the first meiotic metaphase. These polar arrangements of the chromosomes which have been frequently described as fixation artefacts (*cf.* TISCHLER, 1951) have been interpreted by OKSALA (1954) as polarized phases in the development of the meiotic cells. In mitosis polarization is confined to the metaphase and anaphase arrangements of the chromosomes.

At the first division anaphases a number of laggard chromosomes are seen; of these some are univalents, others halves of bivalents or parts of multivalents. The frequency of anaphases with laggards varies in different microspecies between 28 and 61 per cent. Often the laggards divide at the end of

anaphase. Bridges and fragments originating from heterozygous inversions are seen in from 1 to 12 per cent of the anaphase cells. Sometimes the anaphase groups contain different numbers of chromosomes.

At the second division telophase persisting inversion bridges are seen, a part of which originate from the first, another part from the second division. In addition to the four telophase nuclei many cells contain micronuclei, which have arisen from lagging chromosomes. Their frequency varies in the different microspecies. The quality of the pollen is generally poor, varying, however, in the different microspecies. All intermediates between well-developed and quite shrivelled pollen grains are seen. Especially poor is the pollen in the hexaploid *R. auricomus* ssp. *oligandrus*.

The meiotic irregularities characteristic of the *Ranunculus auricomus* group have obviously developed during the apomictic mode of reproduction, as in this case natural selection has much less effect on meiosis than during sexual reproduction. In meiosis of the *auricomus* group more irregularities are found than is usual in pseudogamous plants. The very defective orientation of the chromosomes at the first meiotic metaphase seems to be especially characteristic of this group. The pollen of *R. auricomus* ssp. *oligrandrus* is so poor that autonomous apomixis seems a more probable mode of reproduction than pseudogamy. An investigation of the seed formation of this strain will be the next step in the present study.

All of the microspecies which have been studied are most probably allopolyploid, and owe their origin to hybridization and polyploidization. The very restricted distribution of certain of them suggests a recent origin, and hybridization seems the only plausible explanation. It is probable that there still exist sexual or facultatively apomictic forms in the *auricomus* group, although they have not yet been found. The only known form in this group which obviously is sexual is the Swiss *R. cassubicifolius* (RUTISHAUSER; in NYGREN, 1954). A more extensive investigation may reveal sexual forms also among the Finnish microspecies.

Botanical Institute, University of Helsinki, Finland.

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ARNE LUNDQVIST: Genetics of self-incompatibility in *Festuca pratensis* HUDS.

Self-incompatibility is frequent within the *Gramineae* (EAST, 1940) but the genetic mechanism underlying is not much inquired into. In rye, *Secale cereale*, it is controlled gametophytically by two loci of oppositional alleles (LUNDQVIST, 1954, and unpublished). Thus selection has had an opportunity here to work with allele interaction in the pollen so as to render it consistent with a normal functioning of the incompatibility mechanism. In this case polyploidy will not cause a breakdown of the incompatibility mechanism. As expected, autotetraploid rye is strongly self-incompatible (*e. g.*, LUNDQVIST, 1947). Cases of polyploid grass species with perfect self-incompatibility (*e. g.*, *Dactylis glomerata*) and the selective advantages that may be connected with an apportionment of the incompatibility alleles to two loci (*cf.* LUNDQVIST, 1954) lead one to surmise a wide distribution within the *Gramineae* of the incompatibility system characteristic of rye.

Festuca pratensis, the meadow fescue, is moderately—strongly self-incompatible (*e. g.*, FRANDSEN, 1917; TROLL, 1931; NILSSON, 1934). The species is a diploid perennial with $2n=14$. In the summer of 1954, the author had an opportunity to self some plants of different provenience in a collection of this species kindly put to his disposal by Dr. N. O. BOSEMARK, Lund. The S_1-progeny groups from the two most self-incompatible individuals have been selected for further analysis. Of these two plants, one, 17. Sv. 3: 95, belongs to the »Svalöf late» strain; the other, 32. M. 6, is taken from a Canadian strain named »Mefon».

In the summer of 1955 the siblings of the two S_1-progeny groups were selfed and intercrossed. Owing to the late development and flowering of these plants, characteristic of the yearling stage, backcrosses to the parent plants were not possible. When selfing, a single panicle was isolated; when intercrossing, single panicles from each of the two plants concerned, without preceding emasculation, were brought together in a pergamine bag, in which mutual pollination occurred spontaneously. As the plants were growing in the field, a random distribution of the mating combinations over the whole group has not been possible. The classification of mating results as compatible or not, has not been based on exact countings of seed setting, but in most cases (about 85 %) the classification has been beyond doubt.

On account of the imperfect control of pollination in the sib-matings connected with the almost complete lack of replications of mating combinations, only the positive results are strictly reliable. Poor seed setting may be the result of cross-incompatibility, but also of insufficient pollination. The pro-

portions of compatible combinations obtained will, therefore, constitute minimum values.

26 plants were tested in the progeny group of 17. Sv. 3: 95. Of these, 7 plants turned out to be self-fertile and have thus been excluded. The remaining 19 plants were intercrossed in 74 combinations, 43 of which, or 58,1 %, have been classified as compatible. In order to give an idea of the inter-sib pollination pattern, a particularly complete part of the scheme comprising five of the 19 plants, is presented.

♀ \ ♂	c4	c3	c6	c2	c5
c4	(–)		–	–	–
c3		(–)	–	–	+
c6	+	–	–	–	+
c2	+	+	+	–	+
c5	+	+	+	+	–

The + sign denotes compatible, the — sign incompatible combinations. Of the 18 cross combinations, 11 or 61,1 % are compatible. Assuming that mutual incompatibility will indicate identical, whereas compatibility indicates different genotypes, 4 groups of plants can be distinguished, c3×c6 being incompatible in both directions and thus of identical genotype. All the other combinations are compatible, at least in one direction. The grouping is denoted by the lines in the scheme. Considering the three groups c4, c3 and c6, c2, two successive steps of one-way inter-compatibility are observed: c4 is male compatible with the two other groups, c3 and c6 with c2 only, and c2 is the bottom group, compatible as female only.

The occurrence of more than three inter-compatible groups after selfing, indicates that more than one locus must be concerned in the control of self-incompatibility in *Festuca pratensis*. In an S_1-progeny group, 60,9 % of the inter-sib combinations are expected to be compatible when there are two heterozygous incompatibility loci in the selfed parent plant, the pollen control is gametophytic, and complete incompatibility reaction is not produced unless both factors of the pollen are matched in the style (*cf.* LUNDQVIST, 1954). Denoting the two incompatibility loci as S and Z, respectively, the genotype of the parent plant may be written $S_1S_2Z_3Z_4$. In the progeny group after selfing, 9 different genotypes are possible:

(a) The double heterozygote; $S_1S_2Z_3Z_4$.

(b) Heterozygotes for one locus only; $S_1S_1Z_3Z_4$, $S_2S_2Z_3Z_4$, $S_1S_2Z_3Z_3$, $S_1S_2Z_4Z_4$.

(c) Double homozygotes; $S_1S_1Z_3Z_3$, $S_1S_1Z_4Z_4$, $S_2S_2Z_3Z_3$, $S_2S_2Z_4Z_4$.

A progeny group of this constitution will show two successive steps of one-way inter-compatibility, corresponding to the three possible degrees of heterozygosity:

(a) $S_1S_2Z_3Z_4$ is male compatible with all other genotypes but completely incompatible as female.

(b) A heterozygote for one locus is male compatible with all the other genotypes, except $S_1S_2Z_3Z_4$.

(c) A double homozygote is male incompatible with $S_1S_2Z_3Z_4$, and also with some genotypes heterozygous for one locus.

Thus, c4 in the scheme of plant 17. Sv. 3: 95 may be written as $S_1S_2Z_3Z_4$; c3 and c6 as $S_1S_1Z_3Z_4$; and c2 as $S_1S_1Z_3Z_3$. As can easily be realized, $S_1S_1Z_3Z_3$ is male incompatible with the two other genotypes; $S_1S_1Z_3Z_4$ is male incompatible with $S_1S_2Z_3Z_4$ only; and $S_1S_2Z_3Z_4$ is completely successful as male.

In the progeny group of 32. M. 6, data are less reliable owing to the poor flowering activity of the plants, but even here they clearly speak in favour of more than one locus with oppositional alleles controlling self-incompatibility. Thus, in essential respects self-incompatibility in *Festuca pratensis* seems to follow the same scheme as in rye.

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